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For: CHRONIC RHEUMATOID ARTHRITIS THERAPY CONTAINING IL-6
ANTAGONIST AS EFFECTIVE COMPONENT

TRANSLATOR'S DECLARATION

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Sir:

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- (2) That I translated WO 92/19759, from the Japanese language to the English language;
- (3) That the attached English translation is a true and correct translation of the aforesaid WO 92/19759 to the best of my knowledge and belief; and
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December 1, 2004

Date

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DESCRIPTION

Reshaped Human Antibody to Human Interleukin-6 Receptor

5 Technical Field

The present invention relates to variable regions (V region) of a mouse monoclonal antibody to the human interleukin-6 receptor (IL-6R), human/mouse chimeric antibody to the human IL-6R, and reshaped human antibody comprising a human antibody wherein the complementarity determining regions (CDRs) of the human light chain (L chain) V region and of the human heavy chain (H chain) V region are grafted with the CDRs of a mouse monoclonal antibody to the human IL-6R. Moreover, the present invention provides DNA coding for the above-mentioned antibodies or part thereof. The present invention further provides vectors, especially expression vectors comprising said DNA, and host cells transformed or transfected with said vector. The present invention still more provides a process for production of a chimeric antibody to the human IL-6R, and process for production of a reshaped human antibody to the human IL-6R.

25 Background Art

Interleukin-6 (IL-6) is a multi-function cytokine that is produced by a range of cells. It regulates immune responses, acute phase reactions, and hematopoiesis, and may play a central role in host defense mechanisms. It acts on a wide range of tissues, exerting growth-inducing, growth inhibitory, and differentiation-inducing effects, depending on the nature of the target cells. The specific receptor for IL-6 (IL-6R) is expressed on lymphoid as well as non-lymphoid cells in accordance with the multifunctional properties of IL-6. Abnormal expression of the IL-6 gene has been suggested to be involved in the pathogenesis of a variety of diseases, especially autoimmune diseases, mesangial

proliferative glomerulonephritis, and plasmacytoma/myeloma (see review by Hirano et al., Immunol. Today 11, 443-449, 1990). Human myeloma cells are observed to produce IL-6 and express IL-6R. In 5 experiments, antibody against IL-6 inhibited the in vitro growth of myeloma cells thus indicating that an autocrine regulatory loop is operating in oncogenesis of human myelomas (Kawano et al., Nature, 332, 83, 1988).

10 The IL-6R is present on the surface of various animal cells, and specifically binds to IL-6, and the number of IL-6R molecules on the cell surface has been reported (Taga et al., J. Exp. Med. 196, 967, 1987). Further, cDNA coding for a human IL-6R was cloned and a 15 primary structure of the IL-6R was reported (Yamasaki et al., Science, 241, 825, 1988).

20 □ Mouse antibodies are highly immunogenic in humans and, for this reason, their therapeutic value in humans is limited. The half-life of mouse antibodies in vivo in human is relatively short. In addition, mouse antibodies can not be administered in multiple doses without generating an immune response which not only interferes with the planned efficacy but also risks an adverse 25 allergic response in the patient.

30 To resolve these problems methods of producing humanized mouse antibodies were developed. Mouse antibodies can be humanized in two ways. The more simple method is to construct chimeric antibodies where the V regions are derived from the original mouse monoclonal antibody and the C regions are derived from suitable 35 human antibodies. The resulting chimeric antibody contains the entire V domains of the original mouse antibody and can be expected to bind antigen with the same specificity as the original mouse antibody. In addition, chimeric antibodies have a substantial reduction in the percent of the protein sequence derived from a non-human source and, therefore, are expected to be less immunogenic than the original mouse antibody.

Although chimeric antibodies are predicted to bind antigen well and to be less immunogenic, an immune response to the mouse V regions can still occur (LoBuglio et al., Proc. Natl. Acad. Sci. USA 84, 4220-4224, 1989).

5 The second method for humanizing mouse antibodies is more complicated but more extensively reduces the potential immunogenicity of the mouse antibody. In this method, the complementarity determining regions (CDRs) from the V regions of the mouse antibody are grafted into
10 human V regions to create "reshaped" human V regions. These reshaped human V regions are then joined to human C regions. The only portions of the final reshaped human antibody derived from non-human protein sequences are the CDRs. CDRs consist of highly variable protein sequences.
15 They do not show species-specific sequences. For these reasons, a reshaped human antibody carrying murine CDRs should not be any more immunogenic than a natural human antibody containing human CDRs.

20 As seen from the above, it is supposed that reshaped human antibodies are useful for therapeutic purposes, but reshaped human antibodies to the human IL-6R are not known. Moreover, there is no process for construction of a reshaped human antibodies, universally applicable to any particular antibody. Therefore to construct a fully
25 active reshaped human antibody to a particular antigen, various devices are necessary. Even though mouse monoclonal antibodies to the human IL-6R, i.e., PM1 and MT18, were prepared (Japanese Patent Application No. 2-189420), and the present inventors prepared mouse
30 monoclonal antibodies to the human IL-6R, i.e., AUK12-20, AUK64-7 and AUK146-15, the present inventors are not aware of publications which suggest construction of reshaped human antibodies to the human IL-6R.

35 The present inventors also found that, when the mouse monoclonal antibodies to the human IL-6R were injected into nude mice transplanted with a human myeloma cell line, the growth of the tumor was remarkably

inhibited. This suggests that the anti-human IL-6 receptor antibody is useful as a therapeutic agent for the treatment of myeloma.

Disclosure of Invention

5 Therefore, the present invention is intended to provide a less immunogenic antibody to the human IL-6R. Accordingly, the present invention provides reshaped human antibodies to the human IL-6R. The present invention also provides human/mouse chimeric antibodies 10 useful during the construction of the reshaped human antibody. The present invention further provides a part of reshaped human antibody, as well as the expression systems for production of the reshaped human antibody and a part thereof, and of the chimeric antibody.

15 More specifically, the present invention provides L chain V region of mouse monoclonal antibody to the human IL-6R; and H chain V region of a mouse monoclonal antibody to the human IL-6R.

20 The present invention also provides a chimeric antibody to the human IL-6R, comprising:

(1) an L chain comprising a human L chain C region and an L chain V region of a mouse monoclonal antibody to the IL-6R; and

25 (2) an H chain comprising a human H chain C region and an H chain V region of a mouse monoclonal antibody to the human IL-6R.

30 The present invention also provides CDR of an L chain V region of a mouse monoclonal antibody to the human IL-6R; and CDR of an H chain V region of a mouse monoclonal antibody to the human IL-6R.

The present invention moreover provides a reshaped human L chain V region of an antibody to the human IL-6R, comprising:

35 (1) framework regions (FRs) of a human L chain V region, and

(2) CDRs of an L chain V region of a mouse monoclonal antibody to the human IL-6R; and

a reshaped human H chain V region of an antibody to the human IL-6R comprising:

- (1) FRs of a human H chain V region, and
- (2) CDRs of an H chain V region of a mouse

5 monoclonal antibody to the human IL-6R.

The present invention also provides a reshaped human L chain of an antibody to the human IL-6R, comprising:

- (1) a human L chain C region; and
- (2) an L chain V region comprising human FRs,

10 and CDRs of a mouse monoclonal antibody to the human IL-6R; and

a reshaped human H chain of an antibody to the human IL-6R, comprising:

- (1) a human H chain C region, and

15 (2) an H chain V region comprising a human FRs, and CDRs of a mouse monoclonal antibody to the human IL-6R.

The present invention still more provides a reshaped human antibody to the human IL-6R, comprising:

20 (A) an L chain comprising,

- (1) a human L chain C region, and

(2) an L chain V region comprising human L chain FRs, and L chain CDRs of a mouse monoclonal antibody to the human IL-6R; and

25 (B) an H chain comprising,

- (1) a human H chain C region, and

(2) an H chain V region comprising human H chain FRs, and H chain CDRs of a mouse monoclonal antibody to the human IL-6R.

30 The present invention further provides DNA coding for any one of the above-mentioned antibody polypeptides or parts thereof.

The present invention also provides vectors, for example, expression vectors comprising said DNA.

35 The present invention further provides host cells transformed or transfected with the said vector.

The present invention still more provide a process

for production of a chimeric antibody to the human IL-6R, and a process for production of reshaped human antibody to the human IL-6R.

Brief Description of Drawings

5 Fig. 1 represents expression vectors comprising human cytomegalo virus (HCMV) promoter/enhancer system, useful for the expression of the present antibody peptide.

10 Fig. 2 is a graph showing a result of ELISA for confirmation of an ability of the present chimeric antibody AUK12-20 to bind to the human IL-6R.

Fig. 3 is a graph showing a result of measurement of an ability of the present chimeric antibody AUK12-20 to inhibit the binding of IL-6 to the human IL-6R.

15 Fig. 4 is a graph showing a result of ELISA for binding of the present chimeric antibodies PM1a and PM1b to human IL-6R.

20 Fig. 5 is a graph showing a result of ELISA testing the ability of the present chimeric antibodies PM1a and PM1b to inhibit IL-6 from binding to the human IL-6R.

Fig. 6 is a diagram of the construction of the first version of a reshaped human PM-1 H chain V region.

Fig. 7 is a diagram of the construction of the first version of a reshaped human PM-1 L chain V region.

25 Fig. 8 represents a process for construction of an expression plasmid HEF-12h-gy1 comprising a human elongation factor 1 α (HEF-1 α) promoter/enhancer, useful for the expression of an H chain.

30 Fig. 9 represents a process for construction of an expression plasmid HEF-12k-gk comprising the HEF-1 α promoter/enhancer system, useful for the expression of an L chain.

35 Fig. 10 represents a process for construction of an expression plasmid DHFR-PMh-gy1 comprising HCMV promoter/enhancer and the dihydrofolate reductase (dhfr) gene linked to a defective SV40 promoter/enhancer sequence for amplification, useful for expression of an H

chain.

5 Fig. 11 represents a process for the construction of an expression plasmid DHFR- Δ E-RVh-PM1-f comprising EF1 α promoter/enhancer and dhfr gene linked to a defective SV40 promoter/enhancer sequence for amplification, useful for expression of an H chain.

10 Fig. 12 is a graph showing an ability of version "a" and "b" of the reshaped human PM-1 L chain V region for binding to the human IL-6R.

15 Fig. 13 is a graph showing an ability of version "f" of the reshaped human PM-1 H chain V region plus version "a" of the reshaped PM-1 L chain L chain V region for binding to the human IL-6R.

20 Fig. 14 is a graph showing an ability of version "f" of the reshaped PM-1 H chain V region plus version "a" of the reshaped PM-1 L chain V region to inhibit the binding of IL-6 to the human IL-6R.

25 Fig. 15 represents expression plasmids HEF-V_L-gk and HEF-V_H-g γ 1 comprising a human EF1- α promoter/enhancer, useful for expression of an L chain and H chain respectively.

30 Fig. 16 shows a process for construction of DNA coding for reshaped human AUK 12-20 antibody L chain V region.

35 Fig. 17 is a graph showing results of an ELISA for confirmation of an ability of a reshaped human AUK 12-20 antibody L chain V region to bind to human IL-6R. In the Figure, "Standard AUK 12-20 (chimera) means a result for chimeric AUK 12-20 antibody produced by CHO cells and purified in a large amount.

Fig. 18 is a graph showing a result of an ELISA for an ability of a reshaped human AUK 12-20 antibody (L chain version "a" + H chain version "b") to bind to human IL-6R.

35 Fig. 19 is a graph showing a result of an ELISA for an ability of a reshaped human AUK 12-20 antibody

(L chain version "a" + H chain version "d") to bind to the human IL-6R.

Fig. 20 shows a process for chemical synthesis of a reshaped human sle 1220 H antibody H chain V region.

5 Fig. 21 is a graph showing a result of an ELISA for an ability of a reshaped human sle 1220 antibody (L chain version "a" + H chain version "a") to inhibit the binding of IL-6 to the human IL-6R.

10 Fig. 22 is a graph showing a result of an ELISA for an ability of a reshaped human sle 1220 antibody (L chain version "a" + H chain version "b") to inhibit the binding of IL-6 to the human IL-6R.

15 Fig. 23 is a graph showing a result of an ELISA for an ability of a reshaped human sle 1220 antibody (L chain version "a" + H chain version "c") to inhibit the binding of IL-6 to the human LI-6R.

20 Fig. 24 is a graph showing a result of an ELISA for an ability of a reshaped human sle 1220 antibody (L chain version "a" + H chain version "d") inhibit the binding of IL-6 to the human LI-6R.

Best Mode for Carrying Out the Invention

Cloning of DNA coding for mouse V regions

More specifically, to clone DNA coding for V regions of a mouse monoclonal antibody to a human IL-6R, the 25 construction of hybridoma, which produces a monoclonal antibody to the human IL-6R, is necessary as a gene source. As such a hybridoma, Japanese Patent Application No. 2-189420 describes a mouse hybridoma PM-1 which produces a monoclonal antibody PM1 and the properties 30 thereof. Reference Examples 1 and 2 of the present specification describe the construction process of the hybridoma PM1. The present inventors have constructed hybridomas AUK12-20, AUK64-7, and AUK146-15, each producing a mouse monoclonal antibody to the human IL-6R. 35 The construction process of these hybridomas is described in the Reference Examples 3 of this specification.

To clone desired DNAs coding for V regions, of a mouse monoclonal antibody, hybridoma cells are

homogenized and a total RNA is obtained according to a conventional procedure described by Chirgwin et al., Biochemistry 18, 5294, 1977. Next, the total RNA is used to synthesize single-stranded cDNAs according to the 5 method described by J.W. Larrick et al., Biotechnology, 7, 934, 1989.

Next, a specific amplification of a relevant portion of the cDNA is carried out by a polymerase chain reaction (PCR) method. For amplification of a κ L chain V region 10 of a mouse monoclonal antibody, 11 groups of oligonucleotide primers (Mouse Kappa Variable; MKV) represented in SEQ ID NO: 1 to 11, and an oligonucleotide primer (Mouse Kappa Constant; MKC) represented in SEQ ID NO: 12 are used as 5'-terminal 15 primers and a 3'-terminal primer respectively. The MKV primers hybridize with the DNA sequence coding for the mouse κ L chain leader sequence, and the MKC primer hybridizes with the DNA sequence coding for the mouse κ L chain constant region. For amplification of the H chain 20 V region of a mouse monoclonal antibody, 10 groups of oligonucleotide primers (Mouse Heavy Variable; MHV) represented in SEQ ID NO: 13 to 22, and a oligonucleotide primer (Mouse Heavy Constant MHC) represented in SEQ ID NO: 23 are used as 5'-terminal 25 primers and a 3'-terminal primer, respectively.

Note, the 5'-terminal primers contain the nucleotide sequence GTCGAC near the 5'-end thereof, which sequence provides a restriction enzyme Sal I cleavage site; and the 3'-terminal primer contains the nucleotide sequence CCCGGG near the 5-end thereof, which sequence provides a restriction enzyme Xma I cleavage site. These 30 restriction enzyme cleavage sites are used to subclone the DNA fragments coding for a variable region into cloning vectors.

35 Next, the amplification product is cleaved with restriction enzymes Sal I and Xma I to obtain a DNA fragment coding for a desired V region of a mouse

monoclonal antibody. On the other hand, an appropriate cloning vector such as plasmid pUC19 is cleaved with the same restriction enzymes Sal I and Xma I and the above DNA fragment is ligated with the cleaved pUC19 to obtain 5 a plasmid incorporating a DNA fragment coding for a desired V region of a mouse monoclonal antibody.

The sequencing of the cloned DNA can be carried out by any conventional procedure.

10 The cloning of the desired DNA, and the sequencing thereof, are described in detail in Examples 1 to 3.

Complementarity Determining Regions (CDRs)

15 The present invention provides hypervariable or complementarity determining regions (CDRs) of each V region of the present invention. The V domains of each pair of L and H chains from the antigen binding site.

20 The domains on the L and H chains have the same general structure and each domain comprises four framework regions (FRs), whose sequences are relatively conserved, connected by three CDRs (see Kabat, E.A., Wu, T.T., Bilofsky, H., Reid-Miller, M. and Perry, H., in "Sequences of Proteins of Immunological Interest", US Dept. Health and Human Services 1983). The four FRs largely adopt a β -sheet conformation and the CDRs form loops connecting FRs, and in some cases forming part of, 25 the β -sheet structure. The CDRs are held in close proximity by FRs and, with the CDRs from the other domain, contribute to the formation of the antigen binding site. The CDRs are described in Example 4.

Construction of Chimeric Antibody

30 Prior to designing reshaped human V regions of an antibody to the human IL-6R, it is necessary to confirm that the CDRs to be used actually form an effective antigen binding region. For this purpose, chimeric antibodies were constructed. In addition the amino acid 35 sequences of V regions of mouse anti human IL-6R antibodies predicted from the nucleotide sequences of cloned DNAs of the 4 mouse monoclonal antibodies

described in Example 1 and 2 were compared to each other and to V regions from known mouse and human antibodies. For each of the 4 mouse monoclonal antibodies, a set of typical, functional mouse L and H chain V regions had 5 been cloned. All four mouse anti-IL-6R antibodies, however, had relatively distinct V regions. The 4 antibodies were not simply minor variations of each other. Using the cloned mouse V regions, 4 chimeric anti-IL-6R antibodies were constructed.

10 The basic method for constructing chimeric antibodies comprises joining the mouse leader and V region sequences, as found in the PCR-cloned cDNAs, to human C regions-coding sequence already present in mammalian cell expression vectors. Among said 15 4 monoclonal antibodies, construction of a chimeric antibody from the monoclonal antibody AUK12-20 is described in Example 5.

Construction of a chimeric antibody from the monoclonal antibody PM-1 is described in Example 6. The 20 cDNA coding for the mouse PM-1 κ L chain leader and V region was PCR-subcloned into an expression vector containing a genomic DNA coding for the human kappa C region. The cDNA coding for the mouse PM-1 H chain leader and V regions was PCR-subcloned into an expression 25 vector containing a genomic DNA coding for the human gamma-1 C region. Using specially designed PCR primers, the cDNA coding for the mouse PM-1 V region were adapted at their 5'- and 3'-ends (1) so that they would be easy to insert into the expression vectors and (2) so that 30 they would function properly in these expression vectors. The PCR-modified mouse PM-1 V regions were then inserted into HCMV expression vectors already containing the desired human C regions (Figure 1). These vectors are suitable for either transient or stable expression of 35 genetically-engineered antibodies in a variety of mammalian cell lines.

In addition to constructing a chimeric PM-1 antibody

with V regions identical to the V regions present in mouse PM-1 antibody (version a), a second version of chimeric PM-1 antibody was constructed (version b). In chimeric PM-1 antibody (version b), the amino acid at 5 position 107 in the L chain V region was changed from asparagine to lysine. In comparing the L chain V region from mouse PM-1 antibody to other mouse L chain V regions, it was noticed that the occurrence of an asparagine at position 107 was an unusual event. In 10 mouse κ L chain V regions, the most typical amino acid at position 107 is a lysine. In order to evaluate the importance of having the atypical amino acid asparagine at position 107 in the L chain V region of mouse PM-1 antibody, position 107 was changed to the typical amino 15 acid lysine at this position. This change was achieved using a PCR-mutagenesis method (M. Kamman et al., Nucl. Acids Res. (1989) 17:5404) to make the necessary changes in the DNA sequences coding for the L chain V region.

The chimeric PM-1 antibody version (a) exhibited an 20 activity to bind to the human IL-6R. The chimeric MP-1 antibody version (b) also binds to the human IL-6R as well as version (a). Similarly, from other 2 monoclonal antibodies AUK64-7 and AUK146-15, chimeric antibodies were constructed. All 4 chimeric antibodies bound well 25 to the human IL-6R thus indicating in a functional assay that the correct mouse V regions had been cloned and sequenced.

From the 4 mouse anti-IL-6R antibodies, PM-1 antibody was selected as the first candidate for the 30 design and construction of a reshaped human antibody to the human IL-6R. The selection of mouse PM-1 antibody was based largely on results obtained studying the effect of the mouse anti-IL-6R antibodies on human myeloma tumor cells transplanted into nude mice. Of the 4 mouse 35 anti-IL-6R antibodies, PM-1 antibody showed the strongest anti-tumor cell activity.

Comparison of the V regions from mouse monoclonal

antibody PM-1 to V regions from known mouse and human antibodies

To construct a reshaped human antibody wherein the CDRs of a mouse monoclonal antibody are grafted into a human monoclonal antibody, it is desired that there is high homology between FRs of the mouse monoclonal antibody and FRs of the human monoclonal antibody. 5 Therefore, the amino acid sequences of the L and H chain V regions from mouse PM-1 antibody were compared to all 10 known mouse and mouse V regions as found in the OWL (or Leeds) database of protein sequences.

With respect to V regions from mouse antibodies, the L chain V region of PM-1 antibody was most similar to the L chain V region of mouse antibody musigkcko (Chen, H.-T. et al., J. Biol. Chem. (1987) 262:13579-13583) with a 15 93.5% identity. The H chain V region of PM-1 antibody was most similar to the H chain V region of mouse antibody musigvhr2 (F.J. Grant et al., Nucl. Acids Res. (1987) 15:5496) with a 84.0% identity. The mouse PM-1 20 V regions show high percents of identity to known mouse V regions thus indicating that the mouse PM-1 V regions are typical mouse V regions. This provides further 25 indirect evidence that the cloned DNA sequences are correct. There is generally a higher percent identity between the L chain V regions than between the H chain V regions. This is probably due to the lower amount of diversity generally observed in L chain V regions as compared to H chain V regions.

With respect to V regions from human antibodies, the 30 L chain V region of PM-1 antibody was most similar to the L chain V region of human antibody klhure, also referred to as REI (W. Palm et al., Physiol. Chem. (1975) 356:167-191) with a 72.2% identity. The H chain V region of PM-1 35 antibody was most similar to the H chain V region of human antibody humighvap (VAP) (H.W. Schroeder et al., Science (1987) 238:791-793) with a 71.8% identity. The comparisons to human V regions are most important for

considering how to design reshaped human antibodies from mouse PM-1 antibody. The percent identities to human V regions are less than the percent identities to mouse V regions. This is indirect evidence that the mouse PM-1 V regions do look like mouse V regions and not like human V regions. This evidence also indicates that it will be best to humanize mouse PM-1 V regions in order to avoid problems of immunogenicity in human patients.

The V regions from mouse PM-1 antibody were also compared to the consensus sequences for the different subgroups of human V regions as defined by E. A. Kabat et al. ((1987) Sequences of Proteins of Immunological Interest, Forth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office). The comparisons were made between the FRs of the V regions. The results are shown in Table 1.

Table 1

Percent identities between the FRs from the mouse PM-1 V regions and the FRs from the consensus sequences⁽¹⁾ for the different subgroups of human V regions.

A. FRs in the L chain V regions

	HSGI	HSGII	HSGIII	HSGIV
	70.1	53.3	60.7	59.8

B. FRs in the H chain V regions

	HSGI	HSGII	HSGIII
	44.1	52.9	49.2

35

⁽¹⁾The consensus sequences were taken from the subgroups of human V regions as described in

Kabat et al., (1987).

The FRs of mouse PM-1 L chain V region are most similar to the FRs from the consensus sequence for 5 subgroup I (HSGI) of human L chain V regions with 70.1% identity. The FRs of mouse PM-1 H chain V region are most similar to the FRs from the consensus sequence for subgroup II (HSGII) of human H chain V regions with 52.9% identity. These results support the results obtained 10 from the comparisons to known human antibodies. The L chain V region in human REI belongs to subgroup I of human L chain V regions and the H chain V region in human VAP belongs to subgroup II of human H chain V regions.

From these comparisons to the V regions in human 15 antibodies, it is possible to select human V regions that will be the basis for the design of reshaped human PM-1 V regions. It would be best to use a human L chain V region that belongs to subgroup I (SGII) for the design of reshaped human PM-1 L chain V region and a human 20 H chain V region that belongs to subgroup II (SGII) for the design of reshaped human PM-1 H chain V region.

Design of reshaped human PM-1 variable regions

The first step in designing the reshaped human PM-1 V regions was to select the human V regions that would be 25 the basis of the design. The FRs in the mouse PM-1 L chain V region were most similar to the FRs in human L chain V regions belonging to subgroup I (Table 1). As discussed above, in comparing the mouse PM-1 L chain V region to known human L chain V regions, it was most 30 similar to the human L chain V region REI, a member of subgroup I of human L chain V regions. In designing reshaped human PM-1 L chain V regions, the FRs from REI were used. Moreover the REI FRs were used as starting 35 material for the construction of reshaped human PM-1 L chain V region.

In these human FRs based on REI, there were five differences from the FRs in the original human REI

(positions 39, 71, 104, 105, and 107 according to Kabat *et al.*, 1987; see Table 2). The three changes in FR4 (positions 104, 105, and 107) were based on a J region from another human kappa L chain and, therefore, 5 do not constitute a deviation from human (L. Riechmann *et al.*, *Nature* (1988) 322:21-25). The two changes at positions 39 and 71 were changes back to the amino acids that occurred in the FRs of rat CAMPATH-1 L chain V region (Riechmann *et al.*, 1988).

10 Two versions of reshaped human PM-1 L chain V region were designed. In the first version (version "a"), the human FRs were identical to the REI-based FRs present in reshaped human CAMPATH-1H (Riechmann *et al.*, 1988) and the mouse CDRs were identical to the CDRs in mouse PM-1
15 L chain V region. The second version (version "b") was based on version "a" with only one amino acid change at position 71 in human FR3. Residue 71 is part of the canonical structure for CDR1 of the L chain V region as defined by C. Chothia *et al.*, (*J. Mol. Biol.* (1987)
20 196:901-917). The amino acid at this position is predicted to directly influence the structure of the CDR1 loop of the L chain V region and, therefore, may well influence antigen binding. In the mouse PM-1 L chain V region, position 71 is a tyrosine. In the modified REI
25 FRs used in the design of version "a" of reshaped human PM-1 L chain V region, position 71 was a phenylalanine. In version "b" of reshaped human PM-1 L chain V region, the phenylalanine at position 71 was changed to a tyrosine as found in mouse PM-1 L chain V region.
30 Table 2 shows the amino acid sequences of mouse PM-1 L chain V region, the FRs of REI as modified for use in reshaped human CAMPATH-1H antibody (Riechmann *et al.*, 1988), and the two versions of reshaped human PM-1 L chain V region.

Table 2

Design of two different versions of reshaped human
PM-1 L chain V region.

5

	FR1	CDR1
	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3	3
	12345678901234567890123	45678901234

	V _L PM-1	DIQMTQTSSLSASLGDRV TISC	RASQDISSYLN
	REI	DIQMTQSPSSLSASVGDRV TITC	
10	RV _L a	DIQMTQSPSSLSASVGDRV TITC	RASQDISSYLN
	RV _L b	-----	-----

15

	FR2	CDR2
	4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6
	567890123456789	0123456

	V _L PM-1	WYQQKPDGTIKLLIY	YTSRLHS
	REI	WYQQ <u>K</u> PGKAPKLLIY	
	RV _L a	WYQQKPGKAPKLLIY	YTSRLHS
20	RV _L b	-----	-----

	FR3	CDR3
	6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8	9 0 1 2 3 4 5 6 7
	78901234567890123456789012345678	901234567
25	V _L PM-1 ^z GVPSRFSGSGSGTDYSLTINNLEQEDIATYFC	QQGNTLPYT
	REI GVPSRFSGSGSGTD <u>F</u> FTISSLQPEDIATYYC	
	RV _L a GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	QQGNTLPYT
	RV _L b -----Y-----	-----

30

	FR4
	10
	8901234567
	V _L PM-1
	REI
5	RV _L a
	RV _L b

10 Note: The FRs given for REI are those found in the
reshaped human CAMPATH-1H antibody
(Riechmann *et al.*, 1988). The five underlined
amino acid residues in the REI FRs are those that
differ from the amino acid sequence of human REI
(Palm *et al.*, 1975; O. Epp *et al.*, Biochemistry
15 (1975) 14:4943-4952).

20 The FRs in the mouse PM-1 H chain V region were most
similar to the FRs in human H chain V regions belonging
to subgroup II (Table 1). As discussed above, in
comparing the mouse PM-1 H chain V region to known human
H chain V regions, it was most similar to the human
H chain V region VAP, a member of subgroup II of human
H chain V regions. DNA sequences coding for the FRs in
human H chain V region NEW, another member of subgroup II
25 of human H chain V regions, were used as starting
material for the construction of reshaped human PM-1
H chain V region, and as a base for designing the
reshaped human PM-1 H chain V region.

30 Six versions of reshaped human PM-1 H chain V region
were designed. In all six versions, the human FRs were
based on the NEW FRs present in reshaped human CAMPATH-1H
(Riechmann *et al.*, 1988) and the mouse CDRs were
identical to the CDRs in mouse PM-1 H chain V region.
35 Seven amino acid residues in the human FRs (positions 1,
27, 28, 29, 30, 48, and 71, see Table 3) were identified
as having a possible adverse influence on antigen

binding. In the model of mouse PM-1 V regions, residue 1 in the H chain V region is a surface residue that is located close to the CDR loops. Residues 27, 28, 29, and 30 are either part of the canonical structure for CDR1 of the H chain V region, as predicted by C. Chothia et al., Nature (1989) 34:877-883, and/or are observed in the model of the mouse PM-1 V regions to form part of the first structural loop of the H chain V region (Chothia, 1987). Residue 48 was observed in the model of the mouse PM-1 V regions to be a buried residue. Changes in a buried residue can disrupt the overall structure of the V region and its antigen-binding site. Residue 71 is part of the canonical structure for CDR2 of the H chain V region as predicted by Chothia et al., (1989). The six versions of reshaped human PM-1 antibody incorporate different combinations of amino acid changes at these seven positions in the human NEW FRs (see Table 3).

Table 3

Design of six different versions of reshaped human
PM-1 H chain V region.

5	V _H PM-1	FR1			CDR1
		1 1234567890	2 1234567890	3 1234567890	
	NEW	QVQLQESGPGLVRPSQTL	SLTCTVSGSTFS		SDHAW
	RV _H a	QVQLQESGPGLVRPSQTL	SLTCTVSG Y TFT		SDHAW
	RV _H b	-----	Y--T		-----
10	RV _H c	D-----	Y--T		-----
	RV _H d	-----	Y--T		-----
	RV _H e	D-----	Y--T		-----
	RV _H f	-----	YSIT		-----
15	FR2			CDR2	
	V _H PM-1	4 67890123456789		5 01223456789012345	6 A
	NEW	WIRQFPGNKLEWMG		YIS-YSGITTYNPSLKS	
	RV _H a	WVRQPPGRGLEWIG		YIS-YSGITTYNPSLKS	
20	RV _H b	-----		-----	
	RV _H c	-----		-----	
	RV _H d	-----M-		-----	
	RV _H e	-----M-		-----	
	RV _H f	-----		-----	
25					

		FR3	
	7	8	9
	67890123456789012222345678901234		
		ABC	
	V _H PH-1	RISITRDTSKNQFFLQLNSVTGDTSTYYCAR	
	NEW	RVTMLVDTSKNQFSRLSSVTAADTAVYYCAR	
	RV _H a	RVTMLVDTSKNQFSRLSSVTAADTAVYYCAR	
5	RV _H b	-----R-----	
	RV _H c	-----R-----	
	RV _H d	-----R-----	
		-----R-----	
	RV _H e		
10	RV _H f	-----R-----	

	CDR3	FR4	
	10	11	
	5678900012	34567890123	
	AB		
	V _H PM-1	SLARTTAMDY	WGQGTSVTVSS
15	NEW		WGQGSLVTVSS
	RV _H a	SLARTTAMDY	WGQGSLVTVSS
	RV _H b	-----	-----
	RV _H c	-----	-----
	RV _H e	-----	-----
20	RV _H e	-----	-----
	RV _H f	-----	-----

25 Note: The FRs given for NEW are those found in the
first version of reshaped human CAMPATH-1H
antibody (Riechmann *et al.*, 1988).

Construction of reshaped human PM-1 V regions

30 The first versions of the reshaped human PM-1 L and
H chain V regions were each constructed using a novel
PCR-based method. Essentially, a plasmid DNA coding for
reshaped human V region that already contained suitable

human FRs was modified using PCR primers to replace the CDRs present in the starting reshaped human V region with the CDRs from mouse PM-1 antibody. The starting material for the construction of the reshaped human PM-1 L chain 5 V region was a plasmid DNA containing the reshaped human D1.3 L chain V region. The reshaped human D1.3 L chain V region was constructed based on the FRs present in the human L chain V region of REI. The starting material for the construction of the reshaped human PM-1 10 H chain V region was a plasmid DNA containing the reshaped human D1.3 H chain V region. The reshaped human D1.3 H chain V region was constructed based on the FRs present in the human H chain V region of NEW (M. Verhoeyen et al., Science (1988) 239:1534-1536).

15 Once the starting plasmid DNAs containing the desired human FRs were selected, PCR primers were designed to enable the substitution of the mouse PM-1 CDRs in place of the mouse D1.3 CDRs. For each reshaped human PM-1 V region, three primers containing the DNA 20 sequences coding for the mouse PM-1 CDRs and two primers flanking the entire DNA sequence coding for the reshaped human V region were designated and synthesized. Using the five PCR primers in a series of PCR reactions yielded a PCR product that consisted of the human FRs present in 25 the starting reshaped human V region and the CDRs present in mouse PM-1 V region (see Example 7, and Figures 7 and 8). The PCR products were cloned and sequenced to ensure that the entire DNA sequence of version "a" of reshaped human PM-1 L and H chain V region coded for correct amino 30 acid sequence (SEQ ID NO 55).

The remaining versions of the reshaped human PM-1 V regions were constructed using slight modifications of published PCR-mutagenesis techniques (Kamman et al., 1989). As described for the design of the reshaped human 35 PM-1 V regions, one additional version (version "b") of the reshaped human PM-1 L chain V region was constructed and five additional versions (versions "b", "c", "d",

"e", and "f") of the reshaped human PM-1 H chain V region were constructed. These additional versions contain a series of minor changes from the first versions. These minor changes in the amino acid sequences were achieved 5 using PCR mutagenesis to make minor changes in the DNA sequences. PCR primers were designed that would introduce the necessary changes into the DNA sequence. Following a series of PCR reactions, a PCR product was cloned and sequenced to ensure that the changes in the 10 DNA sequence had occurred as planned. Sequence of the reshaped human PM-1 antibody H chain V region version "f" 15 is shown in SEQ ID NO 54).

Once the DNA sequences of the different versions of reshaped human PM-1 V regions were confirmed by 20 sequencing, the reshaped human PM-1 V regions were subcloned into mammalian cell expression vectors already containing human C regions. Reshaped human PM-1 L chain V regions were joined to DNA sequences coding for human κ C region. Reshaped human PM-1 H chain V regions were 25 joined to DNA sequences coding for human gamma-1 C region. In order to achieve higher levels of expression of the reshaped human PM-1 antibodies, the HCMV expression vectors, as shown in Figure 1, were modified to replace the HCMV promoter-enhancer region 30 with the human elongation factor (HEF-1 α) promoter-enhancer (see Figure 15).

Next, all combinations of the reshaped human L chain versions (a) and (b) with the H chain V region versions (a) to (f) were tested for binding to human IL-6R, and as 35 a result, a reshaped human antibody comprising the L chain version (a) and the H chain version (f) exhibited an ability to bind to IL-6R at a same level as that of chimeric PM-1 (a) (Fig. 13) as described in detail in Example 11.

35 Modifications in the DNA sequences coding for the reshaped human PM-1 V regions to improve the levels of expression.

In reviewing the levels of reshaped human PM-1 antibodies being produced in cos cells, it became apparent that the levels of expression of the reshaped human H chains were always approximately 10-fold lower than the levels of expression of the reshaped human L chains or of the chimeric L or H chains. It appeared that there was a problem in DNA coding for the reshaped human H chain V region that caused low levels of expression. In order to identify whether the lower levels of protein expression were the result of lower levels of transcription, RNA was prepared from cos cells co-transfected with vectors expressing reshaped human PM-1 L and H chains. First-strand cDNA was synthesized as described for the PCR cloning of the mouse PM-1 V regions. Using PCR primers designed to flank the ends of DNA coding for the reshaped human L or H chain V regions, PCR products were generated from the cDNAs that corresponded to reshaped human L chain V region or to reshaped human H chain V region.

For the reshaped human L chain V region, there were two PCR products, one 408 bp long, as expected, and a shorter PCR product 299 bp long. The correct size PCR product made up approximately 90% of the total yield of PCR product and the shorter PCR product made up approximately 10% of the total yield. For the reshaped human H chain V region, there were also two PCR products, one 444 bp long, as expected, and a shorter PCR product 370 bp long. In this case, however, the incorrect, shorter PCR product made up the majority of the total yield of PCR product, approximately 90%. The correct size PCR product made up only approximately 10% of the total yield of PCR product. These results indicated that some of the RNAs coding for the reshaped human V regions contained deletions.

In order to determine which sequences were being deleted, the shorter PCR products were cloned and sequenced. From the DNA sequences, it became clear that

for both the L and H chain V regions specific sections of DNA were being deleted. Examination of the DNA sequences flanking the deleted sequences revealed that these sequences corresponded to the consensus sequences for 5 splice donor-acceptor sequences (Breathnach, R. et al., Ann. Rev. Biochem (1981) 50:349-383). The explanation for the low levels of expression of the reshaped human H chains was that the design of the reshaped human H chain V regions had inadvertently created a rather 10 efficient set of splice donor-acceptor sites. It also appeared that the design of the reshaped human L chain V regions had inadvertently created a rather inefficient set of splice donor-acceptor sites. In order to remove 15 the splice donor-acceptor sites, minor modifications in the DNA sequences coding for versions "a" and "f", respectively, of the reshaped human PM-1 L and H chain V regions were made using the PCR-mutagenesis methods described earlier.

Another possible cause of reduced levels of expression was thought to be the presence of introns in 20 the leader sequences in both the reshaped human L and H chain V regions (SEQ ID NOS: 54 and 55). These introns were originally derived from a mouse mu H chain leader sequence (M.S. Neuberger *et al.*, Nature 1985 25 314:268-270) that was used in the construction of reshaped human D1.3 and V regions (Verhoeven *et al.*, 1988). Since the reshaped human D1.3 was expressed in a mammalian cell vector that employed a mouse immunoglobulin promoter, the presence of the mouse leader 30 intron was important. The leader intron contains sequences that are important for expression from immunoglobulin promoters but not from viral promoters like HCMV (M.S. Neuberger *et al.*, Nucl. Acids Res. (1988) 35 16:6713-6724). Where the reshaped human PM-1 L and H chains were being expressed in vectors employing non-immunoglobulin promoters, the introns in the leader sequences were deleted by PCR cloning cDNAs coding for

the reshaped human V regions (see Example 12).

Another possible cause of reduced levels of expression was thought to be the presence of a stretch of approximately 190 bp of non-functional DNA within the 5 intron between the reshaped human PM-1 H chain V region and the human gamma-1 C region. The reshaped human PM-1 H chain V region was constructed from DNA sequences derived originally from reshaped human B1-8 H chain V region (P.T. Jones et al., Nature (1986) 321:522-525). 10 This first reshaped human V region was constructed from the mouse NP H chain V region (M.S. Neuberger et al., Nature (1985); M.S. Neuberger et al., EMBO J. (1983) 2:1373-1378). This stretch of approximately 190 bp occurring in the intron between the reshaped human 15 H chain V region and the BamHI site for joining of the reshaped human V regions to the expression vector was removed during the PCR cloning of cDNAs coding for the reshaped human V regions.

The DNA and amino acid sequences of the final 20 versions of reshaped human PM-1 L and H chain V regions, as altered to improve expression levels, are shown in SEQ ID NOS: 57 and 56. These DNA sequences code for version "a" of the reshaped human PM-1 L chain V region as shown in Table 2 and version "f" of the reshaped human 25 PM-1 H chain V region as shown in Table 3. When inserted into the HEF-1 α expression vectors (Figure 15), these vectors transiently produce approximately 2 μ g/ml of antibody in transfected cos cells. In order to stably produce larger amounts of reshaped human PM-1 antibody, a 30 new HEF-1 α expression vector incorporating the dhfr gene was constructed (see Example 10, Fig. 11). The "crippled" dhfr gene was introduced into the HEF-1 α vector expressing human gamma-1 H chains as was described for the HCMV vector expressing human gamma-1 H chains. 35 The HEF-1 α vector expressing reshaped human PM-1 L chains and the HEF-1 α -dhfr vector expressing reshaped human PM-1 H chains were co-transfected into CHO dhfr(-) cells.

Stably transformed CHO cell lines were selected in Alpha-Minimum Essential Medium (α -MEM) without nucleosides and with 10% FCS and 500 μ g/ml of G418. Prior to any gene amplification steps, CHO cell lines were observed that 5 produced up to 10 μ g/ 10^6 cells/day of reshaped human PM-1 antibody.

Comparison of V regions from mouse monoclonal antibody AUK 12-20 to V regions from known human antibodies

10 The homology of FRs of κ L chain V region of the mouse monoclonal antibody AUK 12-20 with FRs of human κ L chain V region subgroup (HSG) I to IV, and the homology of FRs of H chain V region of the mouse monoclonal antibody AUK 12-20 with FRs of human H chain 15 V regions subgroup (HSG) I to III are shown in Table 4.

Table 4

20 Percent identities between FRs from the mouse AUK 12-20 V regions and FRs from the consensus sequence for the different subgroups of human V regions

FRs in the L chain V regions

	HSG1	HSG2	HSG3	HSG4
25	65.8	64.0	67.6	67.6

FRs in the H chain V regions

	HSGI	HSGII	HSGIII
30	58.6	53.6	49.1

35 As seen from Table 4, the κ L chain V region of the mouse monoclonal antibody AUK 12-20 is homologous in a similar extent (64 to 68%) with the human κ L chain V region subgroups (HSG) I to IV. In a search of the Data base "LEEDS" for protein, L chain V region of human antibody Len (M. Schneider et al., *Physiol. Chem.* (1975)

366:507-557) belonging to the HSG-IV exhibits the highest homology 68%. On the other hand, the human antibody REI, used for construction of a reshaped human antibody from the mouse monoclonal antibody PM-1 belongs to the HSG I, 5 exhibits a 62% homology with L chain V region of the mouse monoclonal antibody AUK 12-20. In addition, the CDRs in the AUK 12-20 antibody L chain V region particularly CDR2, corresponded better to canonical structures of the CDRs in REI rather than those in LEN.

10 Considering the above, it is not necessary to choose a human antibody used for humanization of the mouse monoclonal antibody AUK 12-20 L chain V region from those antibodies belonging to the HSG IV. Therefore, as in the case of the humanization of the mouse monoclonal antibody 15 PM-1 L chain V region, the FRs of REI are used for humanization of the mouse monoclonal antibody AUK 12-20 L chain V region.

20 As shown in Table 4, H chain V region of the antibody AUK 12-20 exhibits the highest homology with the HSG I. Moreover, in a search of Data base "LEEDS", human antibody HAX (Stollar, B.O. et al., J. Immunol. (1987) 139:2496-2501) also belonging to the HSG I exhibits an about 66% homology with the AUK 12-20 antibody H chain V region. Accordingly, to design reshaped human AUK 12- 25 20 antibody H chain V region, the FRs of the human antibody HAX belonging to the HSG I, and FRs of humanized 425 antibody H chain V region which has FRs consisting of HSGI consensus sequence (Ketteborough C.A. et al., Protein Engineering (1991) 4:773-783) are used. Note, 30 the AUK 12-20 antibody H chain V region exhibits an about 64% homology with version "a" of the humanized 425 antibody H chain V region.

Design of reshaped human AUK 12-20 antibody L chain V regions

35 According to the above reason, reshaped human AUK 12-20 antibody L chain V regions is designed as shown in Table 5 using FRs of the REI.

Table 5

		FR1	CDR1
		1 2 12345678901234567890123	3 4567778901234 ABCD
5	V _L AUK 12-20	DIVLTQSPASLGVSLGQRATISC	RASKSVSTSGYSYMH
	REI	DIQMTQSPSSLSASVGDRVITIC	
	RV _L	DIQMTQSPSSLSASVGDRVITIC	RASKSVSTSGYSYMH
10		FR2	CDR2
		4 567890123456789	5 0123456
	V _L AUK 12-20	WYQQKPGQTPKLLIY	ASNLES
	REI	WYQQTPGKAPKLLIY	
15	RV _L	WYQQKPGKAPKLLIY	ASNLES
		FR3	CDR3
		6 7 8 78901234567890123456789012345678	9 901234567
20	V _L AUK 12-20	GVPARFSGSGSGTDFTLNIHPVEEDAATYYC	QHSRENPYT
	REI	GVPSRFSGSGSGTDYFTISSLQPEDIATYYC	
	RV _L	GVPSRFSGSGSGT <u>DFT</u> FTISSLQPEDIATYYC	QHSRENPYT
		FR4	
25		10 8901234567	
	V _L AUK 12-20	FGGGT K LEIk	
	REI	FGQGT K LQIT	
	RV _L	FGQGT K VEIK	
30	Note:	5 underlined nucleotides are those changed in the design of CAMPATH-1H antibody (see the note of Table 2).	

Design of reshaped human AUK 12-20 antibody H chain V regions

According to the above reason, reshaped human AUK 12-20 antibody H chain V regions are designed using 5 FRs of the reshaped human VHa 425. It is found, however, that nucleotide sequence of DNA coding for reshaped human AUK 12-20 antibody H chain V region thus designed has a sequence well conforming to a splicing donor sequence. Therefore, as in the case of reshaped human PM-1 antibody 10 there is a possibility of an abnormal splicing in the reshaped human AUK 12-20 antibody. Therefore, the nucleotide sequence was partially modified to eliminate the splicing donor-like sequence. The modified sequence is designated as version "a".

15 In addition, version "b" to "d" of the reshaped human AUK 12-20 antibody H chain V region were designed. Amino acid sequences of the versions "a" to "d" are shown in Table 6.

20

Table 6

		FR1	CDR1
		1 2 3	12345
	123456789012345678901234567890		
V _H AUK 12-20	EIQLQQSGPELMKPGASVKISCKASGYSFT		SYYIH
SGI	ZVQLVQSGAEVKKPGXSVXVSCKASGYTFS		
25 RV _H a	QVQLVQSGAEVKKPGASVKVSCKASGYSFT		SYYIH
RV _H b	-----		-----
RV _H c	-----		-----
RV _H d	-----		-----

30

		FR2	CDR2
		4 67890123456789	5 01223456789012345 A
	V _H AUK 12-20	WVKQSHGKSLEWIG	YIDPFNGGTSYNQKFKG
	SGI	WVRQAPGXGLEWVG	
	RV _H a	WVRQAPGQGLEWVG	YIDPFNGGTSYNQKFKG
5	RV _H b	-----	-----
	RV _H c	-----I-	-----
	RV _H d	-----I-	-----
		FR3	
		7 67890123456789012222345678901234	8 9 ABC
10	V _H AUK 12-20	KATLTVDKSSSTAYMHLSSLTSEDSAVYYCAR	
	SGI	RVTXTDXSXNTAYMELSSLRSEDTAVYYCAR	
	RV _H a	RVTMTLDTSTNTAYMELSSLRSEDTAVYYCAR	
	RV _H b	K-----V-----	
	RV _H c	-----	
15	RV _H d	K-----V-----	
		CDR3	FR4
		10 5678900012 AB	11 34567890123
	V _H AUK 12-20	GGN-RF--AY	WGQGTLVTVSA
20	SGI		WGQGTLVTVSS
	RV _H a	GGN-RF--AY	WGQGTLVTVSS
	RV _H b	-----	-----
	RV _H c	-----	-----
	RV _H d	-----	-----
25			

Note: The position where one common amino acid residue is not identified in the HSG I V_H regions (SGI) is shown as "X". Two underlined amino acid residues

are different from those in SGI consensus sequence. For RV_Hb, RV_Hc and RV_Hd, only amino acid residues different from those of RV_Ha are shown.

5 Moreover, version "a" to "d" of reshaped human
AUK 12-20 antibody H chain V region are designed as shown
in Table 7, using FRs of the human antibody HAX (J.
Immunology (1987) 139:2496-2501; an antibody produced by
hybridoma 21/28 cells derived from B cells of a SLE
10 patient; its amino acid sequence is described in Fig. 6
and nucleotide sequence of DNA coding for the amino acid
sequence is shown in Figs. 4 and 6, of this literature).

Table 7

15		FR1			CDR1
		1	2	3	
		12345678901	2345678901	345678901	12345
	V _H AUK 12-20	EIQLQQSGPELMKPGASVKISCKASGYSFT			SYYIH
	SGI	QVQLVQSGAEVKKPGASVKVSCKASGYTFT			
	sle: 1220Ha	QVQLVQSGAEVKKPGASVKVSCKASGYSFT			SYYIH
20	1220Hb	-----	S--		-----
	1220Hc	-----	S--		-----
	1220Hd	-----	S--		-----

		FR2	CDR2
		4 67890123456789	5 0122223456789012345 ABC
	V _H AUK 12-20	WVKQSHGKSLEWIG	YIDP--FNGGTSYNQKFKG
	HAX	WVRQAPGQRLEWMG	
	sle:		
5	1220Ha	WVRQAPGQRLEWMG	YIDP--FNGGTSYNQKFKG
	1220Hb	-----I-	-----
	1220Hc	-----	-----
	1220Hd	-----I-	-----
10			
		FR3	
		7 67890123456789012222345678901234	8 ABC
	V _H AUK 12-20	KATLTVDKSSSTAYMHLSSLTSEDSAVYYCAR	
	HAX	RVTITRDTSASTAYMELSSLRSEDTAVYYCAR	
	sle:		
15	1220Ha	RVTIT <u>D</u> TSASTAYMELSSLRSEDTAVYYCAR	
	1220Hb	-----V-----	
	1220Hc	K----V-----	
	1220Hd	K----V-----	
20			
		CDR3	FR4
		10 5678900012 AB	11 34567890123
	V _H AUK 12-20	GGN-RF--AY	WGQGTLVTVSA
	HAX		WGQGTLVTVSS
	sle:		
25	1220Ha	GGN-RF--AY	WGQGTLVTVSS
	1220Hb	-----	-----
	1220Hc	-----	-----
	1220Hd	-----	-----
30			
	Note:	The two underlined residues in sle1220Ha	

are changes from the HAX FRs. For sle1220Hb, sle1220Hc, and sle1220Hd, only the amino acids in the FRs that differ from those in the HAX FRs are shown.

5

For the production of the present chimeric or reshaped human antibodies to the human IL-6R, any expression system, including eucaryotic cells, for example, animal cells, such as established mammalian cell 10 lines, fungal cells, and yeast cells, as well as procaryotic cells, for example, bacterial cells such as E.coli cells, may be used. Preferably the present chimeric or reshaped human antibodies are expressed in mammalian cells such as cos cells or CHO cells.

15 In such cases, a conventional promoter useful for the expression in mammalian cells can be used. For example, viral expression system such as human cytomegalovirus immediate early (HCMV) promoter is preferably used. Examples of the expression vector 20 containing the HCMV promoter include HCMV-V_H-HC γ 1, HCMV-V_L-HC κ , HCMV-12h-g γ 1, HCMV-12k-g κ and the like derived from pSV2neo, as shown in Fig. 1.

25 Another embodiment of promoter useful for the present invention is the human elongation factor 1 α (HEF-1 α) promoter. Expression vectors containing this promotor include HEF-12h-g γ 1 and HEF-12h-g κ (Figs. 8 and 9), as well as HEF-V_H-g γ 1 and HEF-V_L-g κ (Fig. 15).

30 For gene amplification dhfr in a host cell line, an expression vector may contain a dhfr gene. Expression vectors containing the dhfr gene, are for example, DHFR- Δ E-PMh-g γ 1 (Fig. 10), DHFR- Δ E-RVh-PM1-f (Fig. 11) and the like.

35 In summary, the present invention first provides an L chain V region and an H chain V region of a mouse monoclonal antibody to the human IL-6R, as well as DNA coding for the L chain V region and DNA coding for the H

chain V region. These are useful for the construction of a human/mouse chimeric antibody and reshaped human antibody to the human IL-6R. The monoclonal antibodies are, for example, AUK12-20, PM-1, AUK64-7 and AUK146-15.

5 The L chain V region has an amino acid sequence shown in, for example, SEQ ID NOS: 24, 26, 28 or 30; and the H chain V region has an amino acid sequence shown in SEQ ID NOS: 25, 27, 29, or 31. These amino acid sequences are encoded by nucleotide sequences, for example, shown in

10 SEQ ID NOS: 24 to 31 respectively.

The present invention also relates to a chimeric antibody to the human IL-6R, comprising:

- (1) an L chain comprising a human L chain C region and a mouse L chain V region; and
- 15 (2) an H chain comprising a human H chain C region and a mouse H chain V region. The mouse L chain V region and the mouse H chain V region and DNA encoding them are as described above. The human L chain C region may be any human L chain C region, and for example, is human C_x. The human H chain C region may be any human H chain C region, and for example human C_{y1}.

For the production of the chimeric antibody, two expression vectors, i.e., one comprising a DNA coding for a mouse L chain V region and a human L chain C region under the control of an expression control region such as an enhancer/promoter system, and another comprising a DNA coding for a mouse H chain V region and a human H chain C region under the expression control region such as an enhancer/promotor system, are constructed. Next, the expression vectors are co-transfected to host cells such as mammalian cells, and the transfected cells are cultured in vitro or in vivo to produce a chimeric antibody.

35 Alternatively, a DNA coding for a mouse L chain V region and a human L chain C region and a DNA coding for a mouse H chain V region and a human H chain C region are

introduced into a single expression vector, and the vector is used to transfect host cells, which are then cultured in-vivo or in-vitro to produce a desired chimeric antibody.

5 The present invention further provides a reshaped antibody to the human IL-6R, comprising:

- (A) an L chain comprising,
 - (1) a human L chain C region, and
 - (2) an L chain V region comprising a human L
- 10 chain FRs, and L chain CDRs of a mouse monoclonal antibody to the human IL-6R; and
- (B) an H chain comprising,
 - (1) a human H chain C region, and
 - (2) an H chain V region comprising human H
- 15 chain FRs, and H chain CDRs of a mouse monoclonal antibody to the IL-6R.

In a preferred embodiment, the L chain CDRs have amino acid sequences shown in any one of SEQ ID NOs: 24, 26, 28 and 30 wherein the stretches of the amino acid 20 sequences are defined in Table 9; the L chain CDRs have amino acid sequences shown in any one of SEQ ID NOs: 25, 27, 29 and 31 wherein the stretches of the amino acid sequences are defined in Table 9; human L chain FRs are derived from the REI; and human H chain FRs are derived 25 from the NEW or HAX.

In the preferred embodiment, the L chain V region has an amino acid sequence shown in Table 2 as RV_La; and the H chain V region has an amino acid sequence shown in Table 3 as RV_Ha, RV_Hb, RV_Hc, RV_Hd, RV_He or RV_Hf. The amino 30 acid sequence RV_Hf is most preferable.

For the production of the reshaped human antibody, two expression vectors, i.e., one comprising a DNA coding for the reshaped L chain as defined above under the control of an expression control region such as an 35 enhancer/promoter system, and another comprising a DNA coding for the reshaped human H chain as defined above

under the expression control region such as an enhancer/promoter system, are constructed. Next, the expression vectors are co-transfected to host cells such as mammalian cells, and the transfected cells are 5 cultured in vitro or in-vivo to produce a reshaped human antibody.

Alternatively, a DNA coding for the reshaped human L chain and a DNA coding for the reshaped H chain are introduced into a single expression vector, and the 10 vector is used to transfect host cells, which are then cultured in vivo or in vitro to produce a desired reshaped human antibody.

A chimeric antibody of a reshaped human antibody thus produced can be isolated and purified be a 15 conventional processes such as Protein A affinity chromatography, ion exchange chromatography, gel filtration and the like.

The present chimeric L chain or reshaped human L chain can be combined with an H chain to construct a 20 whole antibody. Similarly, the present chimeric H chain or reshaped human H chain can be combined with an L chain to construct a whole antibody.

The present mouse L chain V region, reshaped human L chain V region, mouse H chain V region and reshaped 25 human H chain V region are intrinsically a region which binds to an antigen, human IL-6R, and therefore considered to be useful as such or as a fused protein with other protein, for preparing pharmaceuticals or diagnostic agents.

Moreover, the present L chain V region CDRs and H chain V region CDRs are intrinsically regions which bind to an antigen, human IL-6R, and therefore considered 30 to be useful as such or as a fused protein with other protein, for preparing pharmaceuticals or diagnostic agents.

DNA coding for a mouse L chain V region of the present invention is useful for construction of a DNA

coding for a chimeric L chain or a DNA coding for a reshaped human L chain.

Similarly, DNA coding for a mouse H chain V region of the present invention is useful for construction of a 5 DNA coding for a chimeric H chain or a DNA coding for a reshaped human H chain. Moreover, DNA coding for L chain V region CDR of the present invention is useful for construction of a DNA coding for a reshaped human L chain V region and a DNA coding for a reshaped human L chain. 10 Similarly, DNA coding for H chain V region CDR of the present invention is useful for construction of a DNA coding for a reshaped human H chain V region and a DNA coding for a reshaped human H chain.

EXAMPLES

15 The present invention will be further illustrated by, but is by no means limited to, the following Examples.

20 Example 1 Cloning of DNA coding for V region of mouse monoclonal antibody to human IL-6R (1)

A DNA coding for the V region of a mouse monoclonal antibody to a human IL-6R was cloned as follows.

1. Preparation of total RNA

25 Total RNA from hybridoma AUK12-20 was prepared according to a procedure described by Chirgwin et al., Biochemistry 18, 5294 (1979). Namely, 2.1×10^8 cells of the hybridoma AUK12-20 were completely homogenized in 20 ml of 4 M guanidine thiocyanate (Fulka). The homogenate was layered over a 5.3 M cesium chloride solution layer 30 in a centrifuge tube, which was then centrifuged in a Beckman SW40 rotor at 31000 rpm at 20°C for 24 hours to precipitate RNA. The RNA precipitate was washed with 80% ethanol and dissolved in 150 μ l of 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA and 0.5% SDS, and after 35 adding Protease (Boehringer) thereon to 0.5 mg/ml, incubated at 37°C for 20 minutes. The mixture was

extracted with phenol and chloroform, and RNA was precipitated with ethanol. Next, the RNA precipitate was dissolved in 200 μ l of 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA.

5 2. Synthesis of single stranded cDNA

To synthesize single stranded cDNA according to a procedure described by J.W. Lerrick et al., *Biotechnology*, 7, 934 (1989), about 5 μ g of the total RNA prepared as described above was dissolved in 10 μ l of 10 50 mM Tris-HCl (pH 8.3) buffer solution containing 40 mM KCl, 6 mM MgCl₂, 10 mM dithiothreitol, 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dCTP, 0.5 mM dTTP, 35 μ M oligo dT primer (Amersham), 48 units of RAV-2 reverse transcriptase (RAV-2: Rous associated virus 2; Amersham) and 25 units of human placenta ribonuclease inhibitor (Amersham), and the reaction mixture was incubated at 37°C for 60 minutes and directly used for the subsequent polymerase chain reaction (PCR) method.

20 3. Amplification of cDNA coding for antibody V region by PCR method

The PCR method was carried out using a Thermal Cycler Model PHC-2 (Techne).

(1) Amplification of cDNA coding for mouse κ light (κ L) chain variable region

25 The primers used for the PCR method were MKV (Mouse Kappa Variable) primers represented in SEQ ID NO: 1 to 11, which hybridize with a mouse κ L chain reader sequence (S.T. Jones et al., *Biotechnology*, 9, 88, 1991), and an MKC (Mouse Kappa Constant) primer represented in SEQ ID NO: 12, which hybridizes with a mouse κ L chain C region (S.T. Jones et al., *Biotechnology*, 9, 88, 1991).

30 First, 100 μ l of a PCR solution comprising 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1 mM dATP, 0.1 mM dGTP, 0.1 mM dCTP, 0.1 mM dTTP, 1.5 mM MgCl₂, 2.5 units of DNA polymerase Ampli Tag (Perkin Elmer Cetus), 0.25 μ M of

each group of MKV primer, 3 μ M MKC primer and 1 μ l of the reaction mixture of the single-stranded cDNA synthesis was heated at an initial temperature of 94°C for 1.5 minutes, and then at 94°C for 1 minute, 50°C for 1 minute and 72°C for 1 minute, in this order. After this temperature cycle was repeated 25 times, the reaction mixture was further incubated at 72°C for 10 minutes.

5 10 (2) Amplification of cDNA coding for mouse H chain V region

As primers for the PCR, MHV (Mouse Heavy Variable) primers 1 to 10 represented in SEQ ID NO: 13 to 22 (S.T. Jones et al., Biotechnology, 9, 88, 1991), and an MHC (Mouse Heavy Constant) primer represented in 15 SEQ ID NO: 23 (S.T. Jones et al., Biotechnology, 9, 88, 1991) were used. Amplification was carried out according to the same procedure as described for the amplification of the κ L chain V region gene in section 3. (1).

20 25 30 35 4. Purification and Digestion of PCR Product

The DNA fragments amplified by the PCR as described above were purified using a QIAGEN PCR product purification kit (QIAGEN Inc. US), and digested with 10 units of restriction enzyme Sal I (GIBCO BRL) in 100 mM Tris-HCl (pH 7.6) containing 10 mM MgCl₂ and 150 mM NaCl, at 37°C for three hours. The digestion mixture was extracted with phenol and chloroform, and the DNA was recovered by ethanol precipitation. Next, the DNA precipitate was digested with 10 units of restriction enzyme Xma I (New England Biolabs), at 37°C for two hours, and resulting DNA fragments were separated by agarose gel electrophoresis using low melting agarose (FMC Bio Products USA).

An agarose piece containing DNA fragments of about 450 bp in length was excised and melted at 65°C for 5 minutes, and an equal volume of 20 mM Tris-HCl (pH 7.5) containing 2 mM EDTA and 200 mM NaCl was added thereon.

The mixture was extracted with phenol and chloroform, and the DNA fragment was recovered by ethanol precipitation and dissolved in 10 μ l of 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA. In this manner, a DNA fragment comprising a gene coding for a mouse κ L chain V region, and a DNA fragment comprising a gene coding for a mouse H chain V region were obtained. Both of the above DNA fragments had a Sal I cohesive end at the 5'-end thereof and an Xma I cohesive end at the 3'-end thereof.

10 5. Ligation and Transformation

About 0.3 μ g of the Sal I - Xma I DNA fragment comprising a gene coding for a mouse κ L chain V region, prepared as described above, was ligated with about 0.1 μ g of a pUC19 vector prepared by digesting plasmid pUC19 by Sal I and Xma I, in a reaction mixture comprising 50 mM Tris-HCl (pH 7.4), 10 mM MgCl₂, 10 mM dithiothreitol, 1 mM spermidine, 1 mM dATP, 0.1 μ g/ml of bovine serum albumin and 2 units of T4 DNA ligase (New England Biolabs), at 16°C for 16 hours.

20 Next, 7 μ l of the above ligation mixture was added to 200 μ l of competent cells of E. coli DH5 α , and the cells were incubated for 30 minutes on ice, for one minute at 42°C, and again for one minute on ice. After adding 800 μ l of SOC medium (Molecular Cloning: A Laboratory Manual, Sambrook et al., Cold Spring Harbor Laboratory Press, 1989), the cell suspension was incubated at 37°C for one hour, and inoculated onto an 2xYT agar plate (Molecular Cloning: A Laboratory Manual, Sambrook et al., Cold Spring Harbor Laboratory Press, 1989), which was then incubated at 37°C overnight to obtain an E. coli transformant. The transformant was cultured in 5 ml of 2xYT medium containing 50 μ g/ml ampicillin, at 37°C overnight, and a plasmid DNA was prepared from the culture according to an alkaline method (Molecular Cloning: A Laboratory Manual, Sambrook et al., Cold Spring Harbor Laboratory Press, 1989). The thus-

obtained plasmid containing a gene coding for a mouse κ L chain V region derived from the hybridoma AUK12-20, was designated p12-k2.

5 According to the same procedure as described above, a plasmid containing a gene coding for a mouse H chain V region derived from the hybridoma AUK12-20 was constructed from the Sal I - Xma I DNA fragment, and designated p12-h2.

10 Example 2 Cloning of DNA coding for V region of mouse monoclonal antibody (2)

Substantially the same procedure as described in Example 1 was applied to the hybridoma PM1, AUK64-7, and AUK146-15, to obtain the following plasmids:

15 a plasmid pPM-k3 containing a gene coding for a κ L chain V region derived from the hybridoma PM1;

a plasmid pPM-h1 containing a gene coding for an H chain V region derived from the hybridoma PM1:

20 a plasmid p64-k4 containing a gene coding for a κ L chain V region derived from the hybridoma AUK64-7;

a plasmid p64-h2 containing a gene coding for an H chain V region derived from the hybridoma AUK64-7;

25 a plasmid p146-k3 containing a gene coding for a κ L chain V region derived from the hybridoma AUK146-15; and

a plasmid p146-h1 containing a gene coding for an H chain V region derived from the hybridoma AUK146-15.

30 Note E. coli strains containing the above-mentioned plasmid were deposited with the National Collections of Industrial and Marine Bacteria Limited under the Budapest Treaty on February 11, 1991, and were given the accession number shown in Table 8.

Table 8

	Plasmid	SEQ ID NO	Accession No.
5	p12 - k2	24	NCIMB 40367
	p12 - h2	25	NCIMB 40363
	pPM - k3	26	NCIMB 40366
	pPM - h1	27	NCIMB 40362
	p64 - k4	28	NCIMB 40368
	p64 - h2	29	NCIMB 40364
	p146 - k3	30	NCIMB 40369
10	p146 - h1	31	NCIMB 40365

Example 3 Sequencing of DNA

15 Nucleotide sequences of a cDNA coding region in the above-mentioned plasmids were determined using a kit, SequenaseTM Version 2.0 (U.S. Biochemical Corp. USA).

20 First, about 3 µg of plasmid DNA obtained as described above was denatured with 0.2 N NaOH, annealed with a sequencing primer, and labeled with ³⁵S-dATP according to a protocol of the supplier. Next, the labeled DNA was applied to 6% polyacrylamide gel containing 8 M urea, and, after electrophoresis, the gel was fixed with 10% methanol and 10% acetic acid, dried, 25 and subjected to autoradiography to determine the nucleotide sequence.

The nucleotide sequence of cDNA coding region in each plasmid is shown in SEQ ID NOS 24 to 31.

Example 4 Determination of CDRs

30 General structures of L chain and H chain V regions are similar each other, wherein 4 frame works (FRs) are linked through 3 super variable regions, i.e.,

complementarity determining regions (CDRs). While amino acid sequences in the FRs are relatively well conserved, amino acid sequences in CDRs are very highly variable (Kabat, E.A. et al., "Sequences of Proteins of Immunological Interest", US Dept. Heath and Human Services 1983).

On the basis of the above-determined amino acid sequences of V regions of mouse monoclonal antibodies to human IL-6R, and according to Kabat, E.A. et al., "Sequences of Proteins of Immunological Interest", US Dept. Health and Human Services 1983, CDRs of each V region of mouse monoclonal antibodies to the human IL-6R were determined as shown in Table 9.

15

Table 9

	plasmid	SEQ ID NO	CDR(1)	CDR(2)	CDR(3)
(Amino acid No.)					
	p12-K2	24	24-38	54-60	93-101
20	p12-h2	25	31-35	50-66	99-105
	pPM-k3	26	24-34	50-56	89-97
	pPM-h1	27	31-36	51-66	99-108
	p64-k4	28	24-38	54-60	93-101
	p64-h2	29	31-35	50-66	99-109
25	p146-k3	30	24-34	50-56	89-97
	p146-h1	31	31-35	50-66	99-106

30

Example 5

Confirmation of expression of cloned cDNA(1) (Construction of Chimeric AUK12-20 antibody)

Construction of Expression Plasmid

A chimeric L chain/H chain was constructed from PCR-cloned cDNAs coding for V regions κ L chain and H chain of AUK12-20. In order to easily join a cDNA coding for the mouse AUK12-20 V region to a DNA coding for a human C region in a mammalian expression vector containing an enhancer and promoter of human cytomegalovirus (HCMV) expression vector, it is necessary to introduce convenient restriction enzyme cleavage sites to the 5'- and 3'- termini of the mouse cDNA.

This modification of the 5'- and 3'- termini was carried out by PCR method. Two sets of primers were designed and synthesized. An L chain V region backward primer (SEQ ID NO: 32) and H chain V region backward primer (SEQ ID NO: 33) were designed so that the primers hybridize with a DNA coding for the beginning of the leader sequence, maintain a DNA sequence essential for efficient translation (Kozak, M., J. Mol. Biol. 196: 947-950, 1987) and form a HindIII site for cloning into the HCMV expression vector. An L chain V region forward primer (SEQ ID NO: 34) and an H chain V region forward primer (SEQ ID NO: 35) were designed so that the primers hybridize with a DNA coding for the terminal portion of the J region, maintain a DNA sequence essential for splicing into the C region and form a Bam HI site for joining to the human C region in the HCMV expression vector.

Following the amplification by the PCR, the PCR product was digested with Hind III and BamHI, cloned into the HCMV vector containing the human κ and $\gamma 1$ chain C regions DNA and sequenced to confirm that errors were not introduced during the PCR amplification. The resulting expression vectors are designated as HCMV-12k-g κ and HCMV-12h-g $\gamma 1$.

The structures of the HCMV expression plasmids are shown in Fig. 1. In the plasmid HCMV-V_L-HC_K, V_L region

may be any mouse L chain V region. In this example, AUK12-20 κL chain V region was inserted to obtain the HCMV-12k. In the plasmid HCMV-V_H-HCγ1, V_H region may be any mouse H chain V region. In this example, AUK12-20 H chain V region was inserted to obtain HCMV-12h-gγ1.

Transient expression in COS cells

To observe transient expression of a chimeric AUK12-20 antibody in COS cells, the expression vectors constructed as described above were tested in the COS cells. The vector DNAs were introduced into COS cells by electroporation using a Gene Pulsar apparatus (Bio Rad). Namely, COS cells were suspended in phosphate-buffered saline (PBS) to a cell concentration of 1×10^7 cells / ml, and to 0.8 ml aliquot of the suspension was added 10 μg (per each plasmid) of DNA. Pulses were applied at 1,900 V and 25 μF.

After recovery period of 10 minutes at a room temperature, the electroporated cells were added to 8 ml of DMEM (GIBCO) containing 10% fetal bovine serum. After incubation for 72 hours, a culture supernatant is collected, centrifuged to eliminate cell debris, and aseptically stored for a short period at 4°C or for a long period at -20°C.

Quantification of chimeric antibody by ELISA

A culture supernatant of the transfected COS cells was assayed by ELISA to confirm the production of chimeric antibody. To detect the chimeric antibody, a plate was coated with goat anti-human IgG whole molecule (Sigma). The plate was blocked, and serially diluted supernatant from the COS cell culture was added to each well. After incubation and washing, alkaline phosphatase-linked goat anti-human IgG (γ-chain specific, Sigma) was added to each well. After incubation and washing, substrate buffer was added thereon. The reaction mixture was incubated, and after termination of the reaction, optical density at 405 nm was measured. As

a standard, purified human IgG (Sigma) was used.

ELISA for confirmation of an ability to bind to human IL-6R

5 A culture supernatant of the transformed COS cells was assayed by ELISA to determine whether the produced antibody can bind to the antigen. To detect the binding to the antigen, a plate was coated with MT18 mouse monoclonal antibody (Reference Example 1), and after blocking with 1% bovine serum albumin (BSA) soluble

10 recombinant human IL-6R (SR 344) was added thereon.

After washing, a serially diluted culture supernatant from the COS cells was added to each well. After incubation and washing alkaline phosphatase-linked goat anti-human IgG was added. The reaction mixture was 15 incubated, and after washing a substrate buffer was added. After incubation, the reaction was terminated, and optical density at 405 nm was measured.

20 A result is shown in Fig. 2. Transfection of gene coding for a chimeric antibody AUK12-20 into COS cells was twice repeated. Both the culture supernatant samples exhibited a strong binding to IL-6R, and optical density at 405 nm was changed in a sample dilution (monoclonal antibody concentration) - dependent manner as shown in Fig. 2 by open circles and closed circles revealing the 25 presence of an antibody to IL-6R in the sample.

Determination of an ability to inhibit the binding to IL-6R with IL-6

30 To determine whether an antibody present in a medium inhibits the binding of IL-6R with IL-6, a plate was coated with MT18 monoclonal antibody (Reference Example 1). After blocking, soluble recombinant human IL-6R (SR 344) was added thereon. After washing, serially diluted sample from COS cell culture was added to each well with biotinylated IL-6.

35 After washing, alkaline phosphatase-linked streptoavidin was added, and after incubation and washing, a substrate buffer was added. The reaction

5 mixture was incubated, and after termination of the reaction, optical density at 405 nm was measured, purified mouse AUK12-20 monoclonal antibody was added as a positive control, and a culture medium from COS cells expressing a non-related antibody was used as a negative control.

10 A result is shown in Fig. 3. A culture supernatant of COS cells transfected with genes coding for chimeric antibody AUK 12-20 exhibited the binding of IL-6R with IL-6 at the highest and second highest concentrations. Namely, as shown by closed circles in Fig. 3, optical density at 405 nm changed in a sample dilution (antibody concentration) dependent manner, revealing the inhibition of the binding to IL-6R with IL-6 by an antibody in the 15 sample. This is further confirmed by substantive conformity with antibody concentration dependent change of the positive control (open circles). Note, the negative control did not exhibit inhibition activity (open triangles).

20 Example 6 Confirmation of expression of cloned cDNA (2) (Construction of chimeric PM-1 antibody)

(Construction of expression vectors)

25 In order to construct vectors expressing chimeric PM-1 antibody, the cDNA clones pPM-k3 and pPM-h1, coding for the mouse PM-1 κL chain and the H chain V regions, respectively, were modified by a PCR technique, and then introduced into the HCMV expression vectors (see Figure 1). The backward primers pmk-s (SEQ NO: 38) for 30 L chain V region and pmh-s (SEQ NO: 40) for H chain V region were designed to hybridize to the DNA sequences coding for the beginning of the leader sequences, and to have Kozak consensus sequence and a HindIII restriction site. The forward primers pmk-a (SEQ No: 36) for 35 L chain V region and pmh-a (SEQ No: 39) for H chain V region were designed to hybridize to the DNA sequences coding for the ends of the J regions, and to have a

splice donor sequence and a BamHI restriction site.

For the kappa L chain V region, two forward primers were synthesized. Although in most kappa L chains lysine at position 107 is conserved, in mouse PM-1 kappa L chain position 107 is an asparagine. In order to investigate the effect of this change on the antigen-binding activity of the chimeric PM-1 antibody, the forward primer pmk-b (SEQ NO: 37) was designed to mutate position 107 from an asparagine to a lysine. Following the PCR reaction, the PCR products were purified, digested with HindIII and BamHI, and subcloned into a pUC19 vector (Yanishe-Perron et al., Gene (1985) 33:103-109). After DNA sequencing, the HindIII-BamHI fragments were excised and cloned into the expression vector HCMV-V_H-HC_{γ1} to obtain HCMV-pmh-g_{γ1} for the chimeric H chain, and into the expression vector HCMV-V_L-HC_k to obtain HCMV-pmka-gk and HCMV-pmkb-gk for the chimeric L chain.

Transfection of cos cells

The vectors were tested in cos cells to look for transient expression of chimeric human PM-1 antibodies. The HCMV-pmh-g_{γ1}, and either HCMV-pmka-gk or HCMV-pmkb-gk were co-transfected into the cos cells by electroporation using the Gene Pulsar apparatus (BioRad). DNA (10 µg of each plasmid) was added to a 0.8 ml aliquot of 1 × 10⁷ cells/ml in PBS. A pulse was delivered at 1,900 volts, 25 microfarads capacitance. After a 10 min recovery period at a room temperature, the electroporated cells were added to 20 ml of Dulbecco's Modified Eagle Medium (DMEM) (GIBCO) containing 10% gamma-globulin-free fetal calf serum. After 72 h incubation, the medium was collected, centrifuged to remove cellular debris, and stored under sterile conditions at 4°C for short periods of time, or at -20°C for longer periods.

Expression and analysis of the chimeric PM-1 antibodies

After 3 days of transient expression, medium from

the cos cells was collected and tested for chimeric PM-1 antibody. The medium was first analyzed by ELISA to determine if human-like antibody was being produced by the transfected cos cells. By using known amounts of purified human IgG as a standard in this assay, it is also possible to estimate an amount of human-like antibody (in this case, chimeric PM-1 antibody) present in the medium from the cos cells. For the detection of human antibody, plates were coated with goat anti-human IgG (whole molecule, Sigma). Following blocking, the samples from cos cells were serially diluted and added to each well. After incubation and washing, alkaline phosphatase-conjugated goat anti-human IgG (gamma chain specific, Sigma) was added. After incubation and washing, substrate buffer was added. After incubation, the reaction was stopped and the optical density at 405 nm measured. Purified human IgG (Sigma) was used as a standard.

The medium from the cos cells transfected with the vectors carrying the chimeric PM-1 genes was positive for the expression of a human-like antibody and the approximate amounts were quantified as described.

Next, the same medium from the cos cells transfected with the vectors carrying the chimeric PM-1 genes was assayed for a an ability to bind to human IL-6R. For the detection of binding to the antigen, plates were coated with MT18 mouse monoclonal anitbody (Reference Example 1), an antibody to the human IL-6R. Following blocking, soluble recombinant human IL-6R (SR344) was added. After washing, the samples were serially diluted and added to each well. After incubation and washing, alkaline phosphatase-conjugated goat anti-human IgG (gamma chain specific sigma) was added. After incubation and washing, substrate buffer was added. After incubation, the reaction was stopped and the optical density at 405 nm measured. There was no standard available for this assay.

Two samples were from transfection with genes coding for a chimeric antibody with V regions identical to those found in mouse PM-1 antibody (chimeric PM-1a antibody, Figure 4). One sample was from transfection with genes 5 coding for a chimeric antibody with a single amino acid change at position 107 in the L chain V region as described above (chimeric PM-1b antibody, Figure 4). All samples showed strong binding to IL-6R that decreased with dilution of the sample. Thus, the chimeric PM-1 10 antibody, as constructed, is functional and can bind well to its antigen. Most importantly, the demonstration of a functional chimeric PM-1 is direct evidence that the correct mouse PM-1 V regions have been cloned and sequenced. The chimeric PM-1 antibody, with either amino 15 acid at position 107 in the L chain V region, bound well to its antigen, IL-6R. It appears that position 107 in the mouse PM-1 L chain V region is not very critical in antigen-binding and that either an asparagine or a lysine at this position will function satisfactorily. Since the 20 mouse PM-1 antibody has an asparagine at this position in its L chain V region, all future work with chimeric PM-1 antibody was done with version a, the version that has V regions identical to those found in mouse PM-1 antibody.

25 In order to stably produce larger amounts of chimeric PM-1 antibody, a new HCMV expression vector incorporating the dhfr gene was constructed. The first step in achieving higher levels of expression of the chimeric PM-1 antibody was to modify the vector HCMV-V_H- 30 HC_{γ1} (Figure 1) so that this vector contained a dhfr gene being expressed by a "crippled" SV40 promoter-enhancer. The SV40 enhancer elements were deleted from the pSV2-dhfr vector (S. Subramani et al., Mol. Cell. Biol. (1981) 1:854-864) and the dhfr gene being expressed by the 35 "crippled" SV40 promoter was inserted into the HCMV-V_H-HC_{γ1} vector in place of the neo gene being expressed by

the SV40 promoter-enhancer. The mouse PM-1 V region was then inserted into this new HCMV-V_H-HC_{V1}-dhfr vector. Construction of the improved expression vector is described in Example 10 in detail.

5 CHO dhfr(-) cells (G. Urlaub et al., Proc. Natl. Acad. Sci. USA (1980) 77:4216-4220) were co-transfected with two plasmid DNAs, the HCMV-V_L-HC_V vector for expressing chimeric PM-1a L chain (HCMV-pmka-gk) and the HCMV-V_H-HC_{V1}-dhfr vector for expressing chimeric PM-1 H chain (DHFR-ΔE PMh-g γ 1; Example 10). DNA (10 μ g/ml of each plasmid) was added to a 0.8 ml aliquot of 1×10^7 cells/ml in PBS. A pulse was delivered at 1900 volts, 25 microfarads capacitance. After a 10 min recovery period at a room temperature, the electroporated cells were 10 added to 10 ml of Alpha minimum essential medium (α -MEM) 15 containing nucleosides and 10% FCS. After overnight incubation, the medium was changed to α -MEM without nucleosides and with 10% FCS and 500 μ g/ml of G418 (GIBCO) for the selection of dhfr $^+$ and neo $^+$ transformed 20 cells. After selection, the selected clones were used for gene amplification. After one round of amplification in 2×10^{-8} M methotrexate (MTX), a cell line (PM1k3-7) 25 was selected that produced approximately 3.9 μ g/10 6 cells/day of chimeric PM-1a antibody.

25 ELISA assay for the ability of chimeric antibodies to inhibit IL-6 from binding to human IL-6R.

Antibodies produced in transfected cos cells or in 30 stable CHO cell lines were assayed to determine whether the antibodies could compete with biotinylated IL-6 for binding to IL-6R. Plates were coated with MT18 mouse monoclonal antibody. Following blocking, soluble 35 recombinant human IL-6R (SR344) was added. After washing, the samples from the cos cells were serially diluted and added together with biotinylated IL-6 to each well. After washing, alkaline phosphatase-conjugated streptavidin was added. After incubation and washing,

substrate buffer was added. After incubation, the reaction was stopped and the optical density at 405 nm measured. The Results are shown in Fig. 5.

5

Example 7 Construction of reshaped human PM-1 antibodies

In order to achieve CDR-grafting more rapidly and efficiently, a method for sequential CDR-grafting by PCR was developed. This method is based on PCR-mutagenesis methods (Kamman *et al.*, 1989).

10

In order to prepare the template DNAs containing the selected human FRs for CDR-grafting, it was necessary to reclone suitable reshaped human V regions into convenient vectors. Plasmid DNAs alys11 and F10 code for reshaped human D1.3 L and H chains and contain the FRs from human REI and NEW, respectively. An approximately 500 bp NcoI-BamHI fragment containing DNA sequence coding for the reshaped human D1.3 L chain V region was excised from alys11 and subcloned into HindIII-BamHI cleaved- pBR327 to obtain a vector V1-lys-pBR327. HindIII-BamHI fragment from the V1-lys-pBR327 was inserted into HindIII-BamHI cleaved pUC19 to obtain a vector V1-lys-pUC19. An approximately 700 bp NcoI-BamHI fragment containing DNA sequence coding for the reshaped human D1.3 H chain V region was excised from F10 and subcloned into the HindIII-BamHI site of pBR327 vector, using a HindIII-NcoI adaptor, yielding Vh-lys-pBR327. A HindIII-BamHI fragment was then excised from this vector and subcloned into HindIII-BamHI cleaved pUC19 vector yielding Vh-lys-pUC19.

25

Note the construction of the plasmid alys11 and the DNA sequence coding for the reshaped human D1.3 L chain V region used in a template is described. The DNA sequence coding for the reshaped human D1.3 H chain V region in the plasmid F10 used as a template is described in V. Verhoeven *et al.*, Science 237:1534-1536 (1988) Fig. 2.

30

Figure 6 diagrams the primers and the PCR reactions

35

used in the construction of the first version of reshaped human PM-1 H chain V region. A backward primer A (APCR1; SEQ NO: 41) and a forward primer E (APCR4; SEQ NO: 42) hybridize to DNA sequences on the vector. Although APCR1 and APCR4 were specifically designed for pUC19 vector, universal M13 sequence primers could be used.

The CDR1-grafting/mutagenic primer B (phv-1; SEQ NO: 43), CDR2-grafting primer C (phv-2; SEQ NO: 44) and CDR3-grafting primer D (phv-3; SEQ NO: 45) were 40-60 bp in length, consisting of DNA sequences coding for CDRs from the mouse PM-1 H chain V region and the human FRs in the template DNA that flank the CDR regions. In the first PCR reaction, the forward primer APCR4 and the backward primer D were used. The first PCR product, which contains the mouse PM-1 CDR3 sequence, was purified and used in the second PCR reaction as a forward primer with primer C as the backward primer. In the same manner, the second and third PCR products, which contain mouse PM-1 CDR2 and CDR3, and all three mouse PM-1 CDRs, respectively, were used as primers in the following PCR step. The fourth PCR product, which has the complete reshaped human PM-1 H chain V region, was purified, digested with HindIII and BamHI, and subcloned into a pUC19 vector for further analysis.

Three mutagenic primers phv-1, phv-2, and phv-3 were synthesized for the construction of reshaped human PM-1 H chain V region. They were purified on 12% polyacrylamide gels containing 8M urea. The mutagenic primer phv-1 was designed not only for mouse PM-1 CDR1-grafting but also for mutations at positions 27 and 30 in human FR1, Ser to Tyr and Ser to Thr, respectively. Each 100 μ l PCR reaction typically contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 250 μ M dNTPs, 50 ng of the template DNA (Vh-lys-pUC19), 2.5 u of AmpliTaq (Perkin Elmer Cetus) and the primers. The first PCR reaction containing 1 μ M of each of the phv-3 and APCR4

primers was carried out, after an initial denaturation at 94°C for 1.5 min, for 30 cycles of 94°C for 1 min, 37°C for 1 min and 72°C for 1 min were repeated. The ramp time between the annealing and synthesis steps was set 5 for 2.5 min. The completion of the last cycle was followed by a final extension at 72°C for 10 min. A 523 bp PCR product was purified using a 1.6% low melting temperature agarose gel and then used as a primer in the second PCR reaction.

10 In the second PCR reaction approximately 1 µg of the purified first PCR product and 25 pmoles of the mutagenic primer phv-2 were used as primers. The PCR conditions were the same as described for the first PCR reaction. In the same manner, a 665 bp PCR product from the second 15 PCR reaction and a 737 bp PCR product from the third reaction were used as primers in the third PCR reaction with the primer phv-1, and in the fourth PCR reaction with the primer APCR1, respectively. A 1.172 kb PCR product from the fourth PCR reaction was purified, 20 digested with HindIII and BamHI, and then an approximately 700 bp fragment containing the reshaped human PM-1 H chain V region was subcloned into a pUC19 vector. Two of four clones sequenced had the DNA sequence coding for the correct amino acid sequence and 25 were designated pUC-RVh-PM1a.

In order to construct other versions of reshaped PM-1 H chain V region, five mutagenic PCR primers were synthesized. Each PCR reaction was essentially carried out under the same condition as described above. For 30 version "b", mutagenic primer phv-m4 (Val-71 to Arg-71; the number is according to Kabat et al; see Table 3) (SEQ ID NO: 46) and APCR4 were used in the first PCR reaction with template DNA, pUC-RVh-PM1a. The PCR product from this first PCR reaction was purified and was used as a 35 forward primer in the second PCR reaction with the primer APCR1. The PCR product from the second PCR reaction was purified using a 1.6% low melting temperature agarose

gel, digested with HindIII and BamHI, and subcloned into a pUC19 vector yielding pUC-RVh-PM1b. In the same manner, version "c" (pUC-RVh-PM1c) was obtained using a mutagenic primer phv-nm (Asp-1 to Gln-1) (SEQ ID NO: 47) and a template pUC-RVh-PM1b; version "d" (pUC-RVh-PM1d) was obtained using a mutagenic primer phv-m6 (Ile-48 to Met-48) (SEQ ID NO: 48) and a template pUC-RVh-PM1b; version "e" (pUC-RVh-PM1e) was obtained using the mutagenic primer phv-m6 and a template pUC-RVh-PM1c; and 10 "version f" (pUC-RVh-PM1f) was obtained using a mutagenic primer phv-m7 (Thr-28 to Ser-28, and Phe-29 to Ile-29) (SEQ ID NO: 49) and a template pUC-RVh-PM1b. Amino acid sequence of the version "f" of the reshaped human H chain V region, and a nucleotide sequence codin therefor is 15 shown in SEQ ID NO: 54.

Figure 7 diagrams the primers and the PCR reactions used in the construction of the first version of reshaped human PM-1 L chain V region. For the construction of the first version of reshaped human PM-1 L chain V region, 20 CDR1-grafting primer pkv-1 (SEQ ID NO: 50), CDR2-grafting primer pkv-2 (SEQ ID NO: 51) and CDR3-grafting primer pkv-3 (SEQ ID NO: 52) were synthesized and purified on a 12% polyacrylamide gel containing 8M urea. PCR reactions were carried out as described above. The 25 first PCR reaction contained 1 μ M of each of the pkv-3 and APCR4 primers. A 350 bp PCR product from the first PCR reaction was purified using a 1.5% low melting temperature agarose gel and used as a forward primer in the second PCR reaction. The PCR product from the second 30 PCR reaction was purified, digested with BamHI and HindIII, and the 500 bp fragment containing the CDR3-grafted DNA was subcloned into a pUC19 vector for DNA sequencing. A plasmid DNA having the correct sequence was identified and used as the template DNA in the 35 following PCR reaction. In the third PCR reaction, 25 pmoles of mutagenic primers pkv-2 and APCR4 were used. The PCR product from the third PCR reaction was purified

and used as a primer, with the primer pkv-1, in the fourth PCR reaction. In the same manner, the PCR product from the fourth PCR reaction was used as a primer, with the APCR1 primer, in the fifth PCR reaction.

5 A 972 bp PCR product from the fifth PCR reaction was purified, digested with BamHI and HindIII, and subcloned into a pUC19 vector for DNA sequencing. A problem was identified in the CDR2 region. Two additional PCR reactions were necessary. In the sixth and seventh PCR
10 reactions, the PCR product from the fifth PCR reaction, as cloned into pUC19 vector, was used as template DNA. In the sixth PCR reaction, the primers were pkv-2 and APCR4. The PCR product from the sixth PCR reaction was purified and used as a primer, with the APCR1 primer, in
15 the seventh PCR reaction. The PCR product of the seventh PCR reaction was purified, digested with BamHI and HindIII, and a 500 bp DNA fragment was subcloned into a pUC19 vector for DNA sequencing. Two of five clones sequenced had the correct DNA sequence. The clone was
20 designated pUC-RV1-PM1a. The sequence is shown in SEQ ID NO: 55.

For the construction of the other version of reshaped human PM-1 L chain V region, a mutagenic primer pkv-m1 (SEQ ID NO: 53) was synthesized. The PCR reactions were essentially as described above. In the first PCR reaction, the mutagenic primer pkv-m1 (Phe-71 to Tyr-71) and the APCR4 primer were used with the template DNA pUC-RV1-PM1a. The PCR product of the first PCR reaction was purified and used as a primer, with the APCR1 primer, in the second PCR reaction. The PCR product of the second PCR reaction was purified, digested with BamHI and HindIII, and subcloned into a pUC19 vector for DNA sequencing. The clone was designated pUC-RV1-PM1b.

Example 8

Construction of vectors that employ the human cytomegalovirus immediate early (HCMV) promoter to express genetically-engineered antibodies in mammalian cells (Fig. 1).

5

The DNA fragments coding for the chimeric PM-1 L and H chain V regions were initially inserted into HCMV vectors (HCMV-V_L-HC_K and HCMV-V_H-HC_{γ1}) designed to express either human kappa L chains or human gamma-1 H chains in mammalian cells (see Figure 1). A detailed description of the construction of the HCMV expression vectors is published in Maeda et al., Human Antibodies and Hybridomas (1991) 2:124-134; C. A. Kettleborough et al., Protein Engineering (1991) 4:773-783. Both vectors are based on pSV2neo (P.J. Southern et al., J. Mol. Appln. Genet. (1982) 1:327-341) and contain the human cytomegalovirus (HCMV) promoter and enhancer (M. Boshart et al., Cell (1985) 41:521-530) for high level transcription of the immunoglobulin L and H chains. The L chain expression vector contains genomic DNA coding for the human kappa C region (T. H. Rabbitts et al., Curr. Top. Microbiol. Immunol. (1984) 113:166-171) and the H chain expression vector contains genomic DNA coding for the human gamma-1 C region (N. Takahashi et al. Cell (1982) 29:671-679). The HCMV expression vectors are versatile and can be used for both transient and stable expression in a variety of mammalian cell types.

Example 9

Construction of vectors that employ the human elongation factor 1α (HEF-1α) promoter to express genetically-engineered antibodies in mammalian cells (Fig. 8 and Fig. 9)

30

The human polypeptide chain elongation factor 1α (HEF-1α) is one of the most abundant proteins. It is expressed in most cells. The transcriptional activity of the human EF-1α promoter-enhancer is about 100-fold

35

stronger than that of the SV40 early promoter-enhancer (D.W. Kim et al., Gene (1990) 91:217-223, and T. Uetsuki et al., J. Biol. Chem. (1989) 264:5791-5798). The 2.5 kb HEF-1 α promoter-enhancer region consists of approximately 5 1.5 kb of DNA flanking the 5'-end of the gene, 33 bp in the first exon, 943 bp in the first intron, and 10 bp of the first part of the 2nd exon. The approximately 2.5 kb HindIII-EcoRI fragment was excised from plasmid DNA pEF321-CAT (D.W. Kim et al., Gene (1990) 91:217-223, and 10 T. Uetsuki et al., J. Biol. Chem. (1989) 264:5791-5798) and cloned into pdKCR vector DNA (M. Tsuchiya et al., EMBO J. (1987) 6:611-616) (K. O'Hare et al., Proc. Natl. Acad. Sci USA Vol. 78, No. 3, 1527-1531, 1981) to replace an approximately 300 bp HindIII-EcoRI fragment containing 15 the SV40 early promoter-enhancer sequence thus yielding pTEF-1. pTEF-1 was digested with EcoRI, filled-in with the Klenow polymerase, and ligated to HindIII linkers. An approximately 1.6 kb HindIII-SmaI fragment was then excised from the modified pTEF-1 vector DNA.

20 Plasmid DNA HCMV-12h-g γ 1 (Δ E2) was constructed from the HCMV-12h-g γ 1 constructed in Example 5 by partially digesting HCMV-12h-g γ 1 with EcoRI, filling-in with klenow polymerase, and self-ligating.

25 The plasmid HCMV-12h-g γ 1 (Δ E2) was digested with EcoRI, filled-in with Klenow polymerase, and digested with HindIII. The resulting approximately 7 kb fragment containing the DNA sequence coding for human gamma-1 C region was ligated to the above-prepared 1.6 kb HindIII-SmaI fragment containing the HEF-1 α promoter- 30 enhancer yielding HEF-12h-g γ 1. The HEF-1 α promoter-enhancer region in this vector was the same as that in pTEF-1 except for 380 bp of DNA flanking the 5'-region. The H chain V region, present as a HindIII-BamHI fragment, was easily interchangeable with other H chain V regions.

35 HindIII-BamHI DNA fragments containing the reshaped H chain V region were excised from the pUC-RVh-PM1a, pUC-

RVh-PM1b, pUC-RVh-PM1c, pUC-RVh-PM1d, pUC-RVh-PM1e, and pUC-RVh-PM1f (Example 7), and inserted into the HindIII-BamHI portion of the HEF-12h-g γ 1 to obtain expression vectors RVh-PM1a, RVh-PM1b, RVh-PM1c, RVh-PM1d, RVh-PM1e and RVh-PM1f, respectively. The expression vectors RVh-PM1a, RVh-PM1b, RVh-PM1c, RVh-PM1d, RVh-PM1e and RVh-PM1f, as well as HEF-PMh-g γ 1 have the reshaped human PM-1 H chain V regions versions "a", "b", "c", "d", "e" and "f", as well as the mouse PM-1 H chain V region, respectively.

To construct the L chain expression vector, HEF-12k-gk, an approximately 3.0 kb PvuI-HindIII fragment containing the HEF-1 α promoter-enhancer region was excised from the HEF-12h-g γ 1 and ligated to an approximately 7.7 kb PvuI-HindIII fragment from the HCMV L chain expression vector HCMV-12k-gk constructed in Example 5 to obtain HEF-12k-gk. As for the H chain expression vector HEF-12h-g γ 1, the L chain V region in HEF-12k-gk, present as a HindIII-BamHI fragment, is easily interchangeable with other L chain V regions.

HindIII-BamHI DNA fragments containing the reshaped human L chain V region were excised from the pUC-RV1-PM1a and pUC-RV1-PM1b (Example 7), and inserted into the HindIII-BamHI portion of the HEF-12k-gk to obtain expression vectors RV1-PM1a and RV1-PM1b, respectively. The expression vectors RV1-PM1a, RV1-PM1b, and HEF-PMk-gk have the reshaped human L chain V regions "a", "b", and the mouse PM-1 L chain V region, respectively.

30 Example 10 Construction of vectors that employ the dihydrofolate reductase (dhfr) gene linked to a defective SV40 promoter-enhancer sequence to achieve high levels of expression of genetically-engineered antibodies in CHO cells (Fig. 10 and Fig. 11).

35 In order to remove the enhancer sequence from the

SV40 early promoter, the plasmid DNA pSV2-dhfr (S.Subramani et al., Mol. Cell. Biol. (1981) 1: 854-864) (ATCC 33694) was digested with SphI and PvuII, filled-in with Klenow polymerase, and self-ligated to yield pSV2-
5 dhfr-ΔE (see Figure 10). An approximately 3.7 kb EcoRI fragment containing the HCMV promoter, the H chain V region, and the human gamma-1 C region was excised from HCMV-PMh-g_γ1 by partially digesting with EcoRI. This fragment was ligated to EcoRI-digested pSV2-dhfr-ΔE to
10 yield DHFR-ΔE-PMh-g_γ1.

A similar vector was constructed based on the H chain expression vector that employs the HEF-1 α promoter-enhancer (see Figure 11). An approximately 3.7 kb EcoRI fragment derived from HCMV-12h-g_γ1 was
15 ligated with EcoRI-digested pSV2-dhfr-ΔE to yield DHFR-ΔE-12h-g_γ1. The BamHI site following the dhfr cDNA sequence in DHFR-ΔE-12h-g_γ1 was removed by partially digesting with BamHI, filling-in with Klenow polymerase, and self-ligating. An approximately 4 kb PvuI-BamHI
20 fragment containing the dhfr cDNA was excised from the modified DHFR-ΔE-12h-g_γ1 DNA and ligated to an approximately 3 kb PvuI-BamHI fragment from RVh-PM1f-4 (constructed in Example 12) to yield DHFR-ΔE-RVh-PM1f.

The improved expression plasmids as prepared above
25 can be used for the production of the reshaped human PH-1 antibodies of the present invention.

Example 11 Expression and analysis of different versions of reshaped human PM-1 antibody

30 The HEF-1 α vectors expressing reshaped human PM-1 L and H chains were co-transfected into cos cells. As a standard control, HEF-1 α vectors expressing chimeric PM-1 L and H chains were also co-transfected into cos cells. After 3 days the medium from the transfected cos cells
35 was collected and analyzed by ELISA (1) for the amount of human IgG antibody present in the supernatant and (2) for the ability of that human IgG to bind to IL-6R. Later

the same samples were also tested by ELISA for the ability of the antibody to inhibit human IL-6 from binding to human IL-6R.

Evaluation of the two versions of reshaped human PM-1 L chain V regions were conducted by co-transfected 5 cos cells with one of the two vectors expressing reshaped human PM-1 L chains (RV1-PM1a or RV1-PM1b) and the vector expressing chimeric PM-1 H chain (HCMV-PMh-g γ 1). Cells were also co-transfected with vectors expressing chimeric 10 PM-1 L and H chains (HCMV-PMka-gk and HCMV-PMh-g γ 1). Data using unpurified cos cell supernatants showed that 15 version "a" of reshaped human PM-1 L chain was equivalent to chimeric PM-1 L chain in assays for binding to IL-6R. Version "b" of reshaped human PM-1 L chain, however, virtually abolished binding to IL-6R (Figure 12). From 20 these results, it was concluded that the change at position 71 in FR3 from phenylalanine (as present in the human REI as modified for CAMPATH-1H) to tyrosine (as present in natural human REI and in mouse PM-1) was very detrimental to the formation of a functional antigen-binding site.

Version "a" of the reshaped human PM-1 L chain V region was selected as the best version. In subsequent experiments evaluating the different versions of reshaped 25 human PM-1 H chain V regions, version "a" of the reshaped human PM-1 L chain V region was always used.

Evaluation of the six versions of reshaped human PM-1 H chain V regions were conducted by co-transfected 30 cos cells with one of the six vectors expressing reshaped human PM-1 H chains (RVh-PM1a, RVh-PM1b, RVh-PM1c, RVh-PM1d, RVh-PM1e or RVh-PM1f) and the vector expressing version "a" of the reshaped human PM-1 L chain (RV1-PM1a). Cells were also co-transfected with vectors expressing chimeric PM-1 L and H chains (HEF-PMK-gk and 35 HEF-PMh-g γ 1). Preliminary data using unpurified cos cell supernatants showed that version "a" of reshaped human PM-1 L chain and version "f" of reshaped human PM-1

H chain were equivalent to chimeric PM-1 L and H chains in assays for binding to IL-6R.

To confirm this preliminary data, chimeric and reshaped human PM-1 antibodies were concentrated and purified from cos cell supernatants using Protein A. Namely the media from cos cells was concentrated using a 100 kd cut-off ultrafiltration device (Amicon). The concentrated media was purified using Protein A agarose (Affi-Gel Protein A MAPSII kit, BioRad). Briefly, the concentrated media was applied to a Protein A agarose column that was equilibrated with five bed volumes of binding buffer. The column was washed with 15 bed volumes of the binding buffer, followed by 5 bed volumes of the elution buffer. The eluate was concentrated and the buffer changed to PBS using a microconcentrator (Centricon 10, Amicon). The purified antibodies were used for further analysis.

The analysis of purified samples of chimeric PM-1 antibody, and reshaped human PM-1 antibodies with version "a" of the L chain V region and versions "a", "b", "c", "d", "e", and "f" of the reshaped human H chain V region was carried out. Version "a" of the L chain plus version "f" of the H chain is clearly the best reshaped human PM-1 antibody. It binds to IL-6R as well as chimeric PM-1 antibody does (Figure 13). It also inhibits human IL-6 from binding to the IL-6R as well as both the mouse and chimeric PM-1 antibodies do (Figure 14).

Example 12 Reconstruction of the reshaped human PM-1 V regions to improve the levels of expression.

In order to remove the introns within the DNA sequences coding for the leader sequences of the reshaped human PM-1 L and H chain V regions (see SEQ ID Nos: 54 and 55), the cDNAs coding for the V regions were recloned using the PCR primers. The L and H chain expression vectors RV1-PM1a and RVh-PM1f were co-transfected into cos cells. After 48 hrs, total RNA was prepared

(Chirgwin *et al.*, Biochemistry (1979) 18:5294-5299) and 5 μ g of total RNA was used for the first strand cDNA synthesis as described for the PCR cloning of mouse antibody V regions. Three PCR primers were designed and 5 synthesized. LEV-P1 (SEQ ID NO: 60) and HEV-P1 (SEQ ID NO: 58) contain the splice donor sequence and the BamHI site and were used as forward primers for the L and H chain V regions, respectively. HEV-P2 (SEQ ID BO: 59) contains the Kozak consensus sequence before the ATG 10 initiation codon and the HindIII site and was used as a backward primer for both the L and H chain V regions. Each 100 μ l PCR reaction contained 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(\text{NH}_4)_2\text{SO}_4$, 2 mM MgSO₄, 0.1% Triton X-100, 0.1 μ g BSA, 250 μ M dNTPs, 2.5 u of Vent DNA 15 polymerase (Biolabs, U.K.), 50% of the first-strand cDNA synthesis reaction and 100 pmoles each of the forward and backward primers. Each PCR tube was overlayed with 50 μ l of mineral oil and then cycled, after an initial melt at 94°C for 1.5 min, at 94°C for 1 min, 50°C for 1 min, and 20 72°C for 1 min, and then at 72°C for 10 min. The 408 bp PCR product containing the L chain V region and the 444 bp PCR product containing the H chain V region were purified using 2.0% low melting temperature agarose gels, digested with BamHI and HindIII, and subcloned into a 25 pUC19 vector to obtain pUC-RV1-PM1a-3 and pUC-RVh-PM1f-3 respectively.

It was revealed that the DNA sequences of the reshaped human PM-1 L and H chain V regions contain inappropriate splice donor and acceptor sites (see SEQ ID 30 NOs: 54 and 55). The sites within the L chain V region are not frequently used (approximately 10% of the mRNA), but the sites within the H chain V region are used frequently (approximately 90% of the mRNA). This aberrant splicing resulted in low levels of expression of 35 the reshaped human PM-1 antibody. In order to avoid aberrant splicing in the V regions, the splice donor

5 sites were removed using a PCR-based method. For the H chain V region, the backward primer NEW-SP1 (SEQ ID NO: 61) and the forward primer NEW-SP2 (SEQ ID NO: 62) were synthesized, changing the DNA sequence TGG GTG AGA to the DNA sequence TGG GTT CGC. The conditions for the PCR reactions were as described above for cDNA cloning except that the template DNA was 50 ng of pUC-RVh-PM1f-3 and the primers were either HEV-P2 and NEW-SP2, or HEV-P1 and NEW-SP1.

10 The PCR products from the two PCR reactions were purified using a 2.0% low melting temperature agarose gel and used in a PCR joining reaction. A 98 μ l PCR reaction containing 0.5 μ g of each of the first PCR products and 5 u of Vent DNA polymerase was incubated at 94°C for 15 2 min, 50°C for 2 min, and 72°C for 5 min, and then 100 pmoles each of HEV-P1 and HEV-P2 primers were added. The PCR tube was overlayed with 30 μ l of mineral oil and subjected to 25 cycles of PCR, at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and then incubated at 72°C 20 for 10 min.

25 In the same manner, the splice donor site in the reshaped human PM-1 L chain V region was removed using PCR primers REI-SP1 (SEQ ID NO: 63) and REI-SP2 (SEQ ID NO: 64) that changed the DNA sequence CAG GTA AGG to the DNA sequence CAG GAA AGG (see). Both PCR products, a 408 bp DNA fragment for the L chain V region and a 444 bp DNA fragment for the H chain V region, were purified 30 using a 2.0% low melting temperature agarose gel, digested with HindIII and BamHI, and subcloned into a pUC19 vector to yield pUC-RV1-PM1a-4 and pUC-RVh-RM1f-4, respectively.

35 RVh-PM1f-4 was constructed by replacing the HindIII-BamHI fragment of RVh-PM1f with the HindIII-BamHI fragment excised from pUC-RVh-PM1f-4. Sequence of reshaped human PM-1 antibody L chain V region version "a" wherein introns have been deleted is shown in SEQ ID NO: 57, and sequence of reshaped human PM-1 antibody

H chain V region version "f" wherein have been deleted is shown in SEQ ID NO: 56.

Example 13 Construction of DNA coding for reshaped human AUK 12-20 antibody L chain V region

5

A process for construction of DNA coding for a reshaped human AUK 12-20 antibody L chain V region is shown in Fig. 16. A gene coding for a human antibody L chain V region is incorporated into pUC19 vector using 10 restriction enzymes HindIII and BamHI. Eight PCR primers (A to H) are prepared, and in the first PCR 4 regions which form a gene coding for the V region are amplified. The primers A and H have homology to DNA sequences on the pUC19 vector. The primers B, C and D are primers of 40 15 to 60 bp length each having a gene sequence of CDR to be grafted, respectively. The primers E, F and G have homology to DNA sequence of 15 to 20 bp length of the 5'-terminus of the primers B, C and D, respectively. Four first PCR use pairs of primers A and E, B and F, C and G, 20 as well as D and H, respectively.

The PCR product A-E encodes FR1, and the PCR product B-F encodes CDR1 and FR2. The 3'-terminal portion of the A-E fragment and the 5'-terminal portion of the B-F fragment have homology in their 15 to 20 bp 25 length, allowing to join these fragments at latter stage. Similarly, the B-F fragment has a homology with the C-G fragment which encodes CDR2 and FR3. The C-G fragment further has a homology with the D-H fragment which encodes CDR3 and FR4. Thus, these 4 fragments can be 30 joined by their mutual homology. After joining these 4 fragments in a PCR reaction mixture, primers A and H are added thereon in the second PCR to amplify a product formed by correct joining of the 4 fragment. The second PCR product thus obtained has three grafted CDRs, and 35 after digestion with HindIII and BamHI, is subcloned into pUC19 vector.

More specifically, as a template, plasmid pUC-RV1-PM1a-4 constructed by inserting a DNA encoding reshaped human PM-1 antibody L chain V region version "a" into plasmid pUC19 was used.

5 The above-mentioned primers A to H have the following sequences.

	Backward Primer	SEQ ID NO.	Forward primer	SEQ ID NO.
	A. REVERSE	83	1220-L16	66
10	B. 1220-L1	65	1220-L2b	68
	C. 1220-L2	67	1220L3b	70
	D. 1220-L3	69	UNIVERSAL	82

15 The backward primers 1220-L1, 1220-L2 and 1220L3 for CDR grafting were purified with 12% polyacrylamide gel containing 8M urea prior to using them.

20 A 100 μ l PCR reaction mixture contained 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(\text{NH}_4)_2\text{SO}_4$, 2 mM MgSO_4 , 0.1% Triton X-100, 1 μ g BSA, 250 μ m dNTPs, 5 units Vent DNA polymerase (BioLabs. U.K.), 50 ng pUC-RV1-PM1a-4 DNA, and 100 p moles each of the forward and backward primers. Each PCR tube was overlaid with 50 μ l of mineral oil, and after an initial denaturation at 94°C for 1.5 minutes, 25 30 cycles of reaction at 94°C for 1 minute, 50°C for 1 minute and 72°C for 1 minute was carried out, followed by an incubation at 72°C for 10 minutes.

30 Each of the PCR products, 252 bp (A-E), 96 bp (B-F), 130 bp (C-G) and 123 bp (D-H) was purified with a 2.0% low melting agarose (FMC, Bio. Products, USA). Namely, an agarose piece containing a DNA fragment was excised, melted at 65°C for 5 minutes, and added to the same volume of 20 mM Tris-HCl (pH 7.5) containing 2 mM EDTA and 200 mM NaCl. The mixture was extracted with phenol 35 and chloroform. The DNA fragment was recovered by an ethanol precipitation, dissolved in 10 mM Tris-HCl

(pH 7.5) containing 1 mM EDTA, and used for PCR joining reaction.

Next, 98 μ l of a PCR reaction mixture containing 0.2 μ g each of the first PCR products and 5 units of Vent 5 DNA polymerase was incubated at 94°C for 2 minutes, 50°C for 2 minutes and 72°C for 5 minutes for a joining reaction. Next, 100 p moles each of the primers A (REVERSE) and H(UNIVERSAL) were added to the reaction mixture to make it to 100 μ l volume, and the reaction 10 mixture was overlaid with 50 μ l of mineral oil and subjected to 30 cycles of a reaction at 94°C for 1 minute, 50°C for 1 minute and 72°C for 1 minute, followed by an incubation at 72°C for 10 minutes.

The second PCR product of 558 bp length containing 15 an L chain V region into which CDRs of the mouse monoclonal antibody AUK 12-20 L chain had been grafted was purified by a 2.0% low melting agarose gel, and after digestion with BamHI and HindIII, subcloned into a pUC19 20 vector to obtain pUC-RLL-1220a, and sequenced. A resulting amino acid sequence of the L chain V region and a nucleotide sequence encoding the amino acid sequence is shown in SEQ ID NO: 71.

Next, for construction of an L chain expression vector, a HindIII-BamHI DNA fragment containing a 25 reshaped human AUK 12-20 antibody L chain V region was excised from the above-mentioned plasmid pUC-RV_L-1220a, and inserted to HindIII-BamHI site of an L chain expression vector HEF-12k-gk to obtain an expression vector RV_L-1220a for reshaped human AUK 12-20 antibody 30 L chain V region version "a".

Example 14. Expression and analysis of reshaped human AUK 12-20 antibody L chain

Transient expression in COS cells

The expression vector RV_L-1220a for reshaped human 35 AUK 12-20 antibody L chain and the expression vector HEF-12h-g γ 1 for chimeric 12-20 antibody H chain (Example 5)

were cotransfected into COS cells to evaluate the reshaped human AUK 1220 antibody L chain version "a". Namely, COS cells were suspended in a phosphate-buffered saline (PBS) at a concentration of 1×10^7 cells 1 ml, 5 and to 0.8 ml of the suspension were added the plasmid DNAs (10 μ g for each plasmid). Pulses were applied to the suspension at an electric capacity of 1,900 V, 25 μ F using a Gene Pulser apparatus (Bio Rad).

After restoraction at a room temperature for 10 minutes, electroporated cells were added to 8 ml of DMEM medium (GIBCO) containing 10% bovine fetal serum. After incubation for 72 hours, supernatant was collected, centrifuged to eliminate cell debris, and stored in an aseptic condition at 4°C for short period or at -20°C for 15 longer period.

Determination of human-like antibody by ELISA

A supernatant of the transfected COS cells was assayed by ELISA and the production of chimeric antibody was confirmed. To detect human-like antibody, a plate 20 was coated with a goat anti-human IgG (whole molecule) (Sigma). After blocking, the supernatant from COS cells was sequentially diluted and added to each well.

The plate was incubated and washed, and an alkaline phosphatase-conjugated goat anti-human IgG (α -chain specific, Sigma) was added thereon. After incubation and washing, a substrate solution was added. After further incubation, the reaction was terminated and an optical density at 405 nm was measured. As a standard, purified IgG (Sigma) was used.

30 ELISA for confirmation of an ability to bind to human IL-6R

A supernatant from the transfected COS cells was assayed by ELISA to determine whether the produced human-like antibody can bind to the antigen, human IL-6R. A 35 plate was coated with a mouse monoclonal antibody MT18 (Reference Example 1). After blocking with 1% BSA,

soluble recombinant human IL-6R (SR 344) was added to the plate. After washing the plate, supernatant from COS cells was sequentially diluted and added to each well of the plate. After inclusion and washing, alkaline 5 phosphatase-conjugated goat anti-human IgG was added to the wells, and after further incubation and washing, a substrate solution was added thereon. After incubation, the reaction was terminated and optical density at 405 nm was measured.

10 A result is shown in Fig. 17. The human-like antibody comprising a combination of a reshaped human AUK 12-20 antibody L chain version "a" and a chimeric 12-20 antibody H chain exhibited a binding ability to IL-6R as strong as chimeric 12-20 antibody. Optical density at 15 405 nm changed in a dilution rate-dependent manner, confirming that the sample contains an antibody to IL-6R. In addition, this result shows that the reshaped human AUK 12-20 antibody L chain version "a" has an antigen binding ability as high as chimeric AUK 12-20 20 antibody L chain.

Example 15. Construction of gene coding for reshaped human AUK 12-20 antibody H chain using HSGI consensus sequence

According to the same procedure as described in 25 Example 13. CDRs of AUK 12-20 antibody H chain V region were grafted into the reshaped human $V_{H}a425$ containing HSG I consensus sequences as its FRs (Kettleborough et al., Protein Engineering (1991) 4:773-783). First, a HindIII-BamHI DNA fragment encoding the reshaped human 30 $V_{H}a425$ (Fig. 3 in the literature) was excised from a plasmid HCMV-RV $V_{H}a$ -425- γ 1 and subcloned at HindIII-BamHI sites in pUC 19 vector to obtain pUC-RV H -425a, which was then used as a template. 8 PCR primers (A1 to H1) were synthesized. The primer 1220-H1 was designed to 35 graft CDR1 and to induce a mutation from T-28 to S-28, and the primer 1220-H3 was designed to graft CDR3 and to

induce a mutation from S-94 to R-94. The primers 1220-H1, 1220-H2 and 1220-H3 were purified using a 12% polyacrylamide gel containing 8 M urea prior to using them. Nucleotide sequence of each primer was as follow.

5

	Backward primer	SEQ ID NO.	Forward primer	SEQ ID NO.
	A1. REVERSE	83	E1. 1220-H1b	73
10	B1. 1220-H1	72	E1. 1220-H2b	75
	C1. 1220-H2	74	G1. 1220-H3b	77
	D1. 1220-H3	76	H1. UNIVERSAL	82

15 Condition of PCR was the same as that described in Example 13, except that the pUC-RV_H-425a was used as a template DNA and the above-mentioned primers were used for grafting H chain CDRs. Primer pairs of A1 and E1, B1 and F1, C1 and G1, as well as D1 and H1 were used to carry out first PCR reactions, and the respective first PCR products, 186 bp (A1-E1), 75 bp (B1-F1), 173 bp (C1-G1) and 105 bp (D1-H1) were purified with 2.0% low melting agarose gel, and used in subsequent second PCR joining reaction. According to the condition described 20 in Example 13, 0.2 µg each of the first PCR products were used to carry out the second PCR reaction (including PCR joining reaction) to obtain a PCR product of 495 bp containing DNA coding for a human H chain V region into which mouse AUK 12-20 antibody H chain V region CDRs had 25 been grafted, and the PCR product was purified using 2.5% low melting agarose gel. After digesting the PCR product with BamHI and HindIII, resulting BamHI-HindIII DNA fragment was subcloned into pUC19 and sequenced to obtain pUC-RV_H-1220a.

30 35 It was revealed that DNA sequence coding for reshaped human AUK 12-20 antibody H chain V region contains a sequence well conforming to a splicing donor

sequence, which may cause an abnormal splicing which was troublesome in the production of the reshaped human PM-1 antibody. Therefore, this DNA sequence was modified by PCR. Mutagenetic primers, SGI-SP1 (SEQ ID NO: 97) and 5 SGI-SP2 (SEQ ID NO: 98) were synthesized. These primers convert the DNA sequence AAG GTG AGC to the DNA sequence AAA GTC AGC. Condition of PCR reaction was same as described above, except that 50 ng of pUC-RV_H-1220a was used as a template DNA, and the SGI-SP1 and UNIVERSAL 10 (SEQ ID NO: 82), or the SGI-SP2 and REVERS (SEQ ID NO: 83) were used as primers.

PCR products from two PCR reactions were purified by 2% low melting agarose gel and used in a PCR joining reaction. 98 μ l of PCR reaction mixture containing 15 0.2 μ g each of the first PCR products and 5 units of Vent DNA polymerase was incubated at 94°C for 2 minutes, at 55°C for 2 minutes and at 72°C for 5 minutes for a joining reaction. Next, 100 pmoles each of UNIVERSAL and REVERSE primers were added to the reaction mixture, which 20 was then overlaid with 50 μ l of mineral oil and subjected to 30 cycles of second PCR reaction consisting of incubations at 94°C for 1 minutes, at 50°C for 1 minute and at 72°C for 1 minute, followed by an incubations at 72°C for 10 minutes. DNA fragment of 495 bp obtained in 25 the second PCR was purified by a 2.0% low melting agarose gel, and subcloned into pUC19 vector and sequenced to obtain pUC-RV_H-1220a-2.

Next, HindIII-BamHI DNA fragment containing DNA 30 coding for reshaped human AUK 12-20 antibody H chain V region was excised from the pUC-RV_H-1220a-2, and inserted at HindIII-BamHI sites of an H chain expression vector HEF-12h-g γ 1 to obtain an expression vector RV_H-1220a for the reshaped human AUK 12-20 antibody H chain version "a".

35 For construction of genes coding for reshaped human AUK 12-20 antibody H chain V region versions "b" to "d",

two pairs of mutagenic primers were synthesized. Each PCR reaction was carried out under substantially the same condition as described above. For construction of version "b", in two first PCR reactions, either UNIVERSAL 5 primer (SEQ ID NO: 82) and mutagenic primer 120H-ml (SEQ ID NO: 78), or REVERSE primer (SEQ ID NO: 83) and mutagenic primer 1220H-mlb (SEQ ID NO: 79), as well as pUC-RV_H-1220a as a template were used. The first PCR products of 202 bp and 323 bp were purified by a 2.0% low 10 melting agarose gel, and used in second PCR (including PCR joining reaction) under the same condition as described above to obtain a 495 bp product (version "b"). The product was digested with HindIII and BamHI, and subcloned into pUC19 vector to obtain pUC-RV_H-1220b.

15 Similarly, mutagenic primer 1220H-m2 (SEQ ID NO: 80), 1220H-m2b (SEQ ID NO: 81) and a template pUC-RV_H-1220a were used in a PCR to obtain a PCR product 20 (version "c"). The product was digested with HindIII-BamHI and inserted at HindIII-BamHI sites of pUC19 vector to obtain pUC-RV_H-1220c. Moreover, mutagenic primers 1220H-mla (SEQ ID NO: 78), 1220H-mlb (SEQ ID NO: 79), and a template pUC-RV_H-1220c were used to obtain a PCR 25 Product (version "d"), which was then digested with HindIII and BamHI and inserted into HindIII-BamHI sites of pUC19 vector to obtain pUC-RV_H-1220d.

30 Note, an amino acid sequence of the reshaped human AUK 12-20 antibody H chain V region version "b" and a nucleotide sequence coding therefor in the plasmid pUC-RV_H-1220b is shown in SEQ No. 84; and an amino acid sequence of the reshaped human AUK 12-20 antibody H chain V region version "d" and a nucleotide sequence coding therefor in the plasmid pUC-RV_H-1220d is shown in SEQ ID NO: 85.

35 Next, to construct the expression vectors, HindIII-BamHI fragments containing a reshaped human AUK 12-20 antibody H chain V region were excised from pUC-RV_H-

1220b, pUC-RV_H-1220c and pUC-RV_H-1220d and inserted into HindIII-BamHI sites of H chain expression vector HEF-12h-g γ 1 to obtain RV_H-1220b, RV_H-1220c and RV_H-1220d respectively.

5 Example 16. Expression and analysis of various
 versions of reshaped human AUK 12-20
 antibody.

COS cells were cotransfected with one of 4 expression vectors for reshaped human AUK 12-20 antibody H chain (RV_H-1220a, RV_H-1220b, RV_H-1220c or RV_H-1220d) and an expression vector VR_L-1220a to evaluate 4 versions of the reshaped human AUK 12-20 antibody H chain V region. For reference, COS cells were cotransfected with expression vectors for chimeric 12-20 antibody L chain and H chain (HEF-12h-g γ 1 and FEF-12-gk). In an assay for binding to the human IL-6R, a reshaped human AUK 12-20 antibody consisting of reshaped human AUK 12-20 antibody L chain and reshaped human AUK 12-20 antibody H chain version "b", and a reshaped human AUK 12-20 antibody consisting of reshaped human AUK 12-20 antibody L chain and reshaped human AUK 12-20 antibody H chain version "d" shows good binding as well as chimeric 12-20 antibody. These results are shown in Figs. 18 and 19.

20 Example 17. Construction of gene coding for
 reshaped human sle 1220 antibody
 H chain using human antibody HAX

A human antibody having the highest homology with the mouse monoclonal antibody AUK 12-20 H chain V region 30 is HAX (J. Immunology (1987) 139:2496-2501; an antibody produced by hybridoma 21/28 derived from B cells of an SLE patient; its amino acid sequence is shown in Fig. 6, and nucleotide sequence therefor is shown in Figs. 4 and 5 of this literature), according to a protein data base 35 "Leeds". Reshaped human sle 1220H antibody H chain V region was constructed using FRs of the antibody HAX

and CDRs of mouse monoclonal antibody AUK 12-20 H chain V region.

An entire DNA coding for a reshaped human sle 1220 H antibody H chain V region version "a" was chemically synthesized. DNA coding for sle 1220 H antibody H chain V region of an entire length 439 bp was designed by dividing the DNA into 6 oligonucleotides of 90 to 94 bp length overlapping each other by 21 bp (sle 1220 h 1 to 6; SEQ ID NOS: 86 to 91, respectively). In designing the oligonucleotides, secondary structure was tested and for sites having structural problems the third nucleotide in a codon was changed without change of amino acid encoded thereby. The relationship of these oligonucleotides and a process for construction of double-stranded synthetic DNA are shown in Fig. 20.

The reaction shown in Fig. 20 is carried out using PCR technique. Namely, 6 synthetic oligonucleotides were added to a single PCR reaction tube to carry out the first PCR reaction, thereby two oligonucleotides are annealed and extended, and further 4 oligonucleotides or an entire oligonucleotide are obtained.

Next, terminal primers A (SEQ ID NO: 92) and B (SEQ ID NO: 93) are added to carry out the second PCR reaction, wherein only a correct oligonucleotide having an entire length can be amplified. The resulting product is digested with BamHI and HindIII, and subcloned into pUC19 vector, followed by sequencing.

More specifically, 98 μ l of a reaction mixture containing 100 mM tris-HCl (pH 8.5), 50mM KCl, 0.1mM dATP, 0.1mM dGTP, 0.1mM dCTP, 0.1mM dTTP, 1.5mM MgCl₂, and 2.5 U of DNA polymerase AmpliTaq (Perkin Elmer Cetus) as well as 5 pmoles each of the oligonucleotides was denatured at 94°C for 1.5 minutes and subjected to 3 cycles of reaction by incubation at 92°C for 3 minutes, 50°C for 2 minutes and 72°C for 5 minutes, followed by an incubation at 72°C for 10

minutes. One μ l each of 50 mM terminal primers A and B were added to the reaction mixture, which was then overlaid with 80 μ l of mineral oil, and after denaturation of 94°C for 1.5 minutes, subjected to 30 cycles of reaction by incubation at 94°C for 1 minute, 50°C for 1 minute and at 72°C for 1 minute, followed by an incubation at 72°C for 10 minutes. The PCR product of 439 bp was purified by a 1.5% low melting agarose gel, digested with restriction enzymes BamHI HindIII, and 10 subcloned into pUC19 vector, followed by confirmation of sequence. A clone thus obtained was designated pUC-RV_H-sle 1220Ha. An amino acid sequence of reshaped human sle 1220H antibody H chain V region version "a" and a nucleotide coding therefor in the plasmid pUC-RV_H-sle 1220Ha are shown in SEQ ID NO: 94.

15 Next, HindIII-BamHI DNA fragment containing a gene coding for reshaped human 12-20 (sle 1220H) antibody H chain V region was excised from the pUC-RV_H-sle 1220Ha and inserted at HindIII-BamHI sites of an H chain 20 expression vector HEF-12h-gyl to obtain RV_H-sle 1220Ha.

For construction of version "b" to "d" of reshaped human sle 1220H antibody H chain V region, two mutagenic primers sle 1220Hml (SEQ ID NO 95) and sle 1220Hm2 (SEQ ID NO: 96) were synthesized. In each PCR, Vent DNA 25 polymerase and reaction mixture composition described in Example 13 were use. In each PCR reaction, a reaction mixture containing pUC-RV_H-sle 1220Ha as template, 50 pmoles of a mutagenic primer sle 1220Hml or sle 1220Hm2, and 50 pmoles of the terminal primer B was denatured at 30 94°C for 1.5 minutes, and subjected to 30 cycles of reaction by incubation at 94°C for 1 minute, at 50°C for 1 minute and at 72°C for 1 minute, followed by an incubation at 72°C for 10 minutes. The product of 235 bp or 178 bp was purified by a 1.5% low melting agarose gel 35 to use as a primer in the second PCR reaction. Namely the second PCR reaction was carried out using 50 pmoles

of the terminal primer A, 0.2 μ g of the PCR product and pUC-RV_H-sle 1220Ha as a template, and resulting product of 439 bp was purified by a 1.5% low melting agarose gel, digested with BamHI and HindIII, and subcloned into pUC19 vector to obtain pUC-RV_H-sle 1220Hb or pUC-RV_H-sle 1220Hc, which encodes reshaped human sle 1220 antibody H chain V region version "b" or "c", respectively.

A DNA coding for reshaped human sle 1220 H antibody H chain V region version "d" was constructed also follow. As a template pUC-RVh-sle 1220Hb was used. 50 pmoles each of a mutagenic primer sle 1220Hm2 and the terminal primer B was used to carry out 30 cycles of the first PCR reaction. Resulting 176bp PCR product was purified on a 1.6% low melting agarose gel to use as a primer in the second PCR. This primer and 50p moles of the terminal primer A was used in the second PCR to obtain a 439 bp DNA fragment. The PCR product thus obtained was purified, digested with BamHI and HindIII, and subcloned into pUC 19 vector to obtain pUC-RV_H-sle 1220Hd.

Next, to construct expression vectors for various versions of reshaped human sle 1220H antibody H chain V region, BamHI-HindIII fragments containing a DNA encoding reshaped human sle 1220 antibody H chain V region were excised from pUC-RV_H-sle 1220Hb, pUC-RV_H-sle 1220Hc and pUC-RV_H-sle 1220Hd, and inserted into HindIII-BamHI sites of the H chain expression vector HEF-12h-g γ 1 to obtain expression vectors RV_H-sle 1220Hb, RV_H-sle 1220Hc and RV_H-sle 1220Hd respectively.

Each of four vectors expressing reshaped human sle 1220H antibody H chain (RV_H-sle 1220Ha, RV_H-sle 1220Hb and RV_H-sle 1220Hc or RV_H-sle 1220Hd) and the vector RV_L-1220a expressing reshaped human AUK 12-20 antibody L chain were cotransfected to COS cells to evaluate the four versions of the reshaped human sle 1220H antibody H chain V region for an ability to

inhibit the binding of IL-6 to IL-6R. Results is shown in Figs. 21 to 24. Note, these result were obtained after purifying the produced antibodies by protein A.

As seen from the above, according to the present invention, in a chimeric L chain or a reshaped human L chain, or a chimeric H chain or a reshaped human H chain, and especially in RF, one or more than one amino acid can be replaced with other amino acid maintaining an ability to bind to human IL-6R. Therefore, the present invention includes chimeric antibody and reshaped human antibody, chimeric L chain and reshaped human L chain, chimeric H chain and reshaped human H chain, reshaped L chain V region, and reshaped H chain V region, wherein one or more than one amino acid is replaced with other as well as DNA coding therefor, as far as they maintain their native property.

Starting hybridomas used in the present invention were constructed as follows.

	<u>Reference Example 1</u>	<u>Construction of Hybridoma</u>
20		<u>MT18</u>

To construct a hybridoma producing monoclonal antibody to human IL-6R, as an immunogen, a mouse T cell line expressing human IL-6R on the cell surface was constructed as follows. Namely, a plasmid pBSF2R.236 disclosed in Japanese Patent Application No. H1-9774 and pSV2neo was transfected into a mouse T cell line CTLL-2 (ATCC TIB214) according to a conventional procedure, and the resultant transformant was screened using G418 according to a conventional procedure to obtain a cell line expressing about 30,000 IL-6Rs per cell. This cell line was designated CTBC3.

The CTBC3 cells were cultured in RPMI 1640 according to a conventional procedure, the cultured cells were washed four times with PBS buffer, and 1×10^7 cells were intraperitoneally injected to C57BL/6 mice for immunization. The immunization was carried out once a

week for 6 weeks.

Spleen cells were obtained from the immunized mice and fused with myeloma P3U1 cells using polyethylene glycol according to a conventional procedure, and the 5 fused cells were screened as follows. The IL-6R negative human T cell line JURKAT (ATCC CRL 8163) was co-transfected with the plasmids pBSF2R.236 and pSV2neo, and transformed cells were screened to obtain a cell line expressing about 100,000 IL-6Rs per cell. The cell line 10 was designated NJBC8. A hybridoma cell clone producing an antibody which recognized NP40-lysed NJBC8 but did not recognize NP40-lysed JURKAT was cloned and designated MT18. The hybridoma MT18 was deposited with the 15 Fermentation Research Institute Agency of Industrial Science and Technology (FRI), under the Budapest Treaty, as FERM BP-2999 on July 10, 1990.

Reference Example 2

Construction of Hybridoma
PM1

To construct a hybridoma producing monoclonal 20 antibody to the IL-6R, as an antigen, human IL-6R was extracted as follows. 3×10^9 human myeloma U266 cells (IL-6R-producing cells) were lysed in 1 ml of 1% digitonin, 10 mM triethanolamine buffer (pH 7.4), 0.15 M NaCl and 1 mM PMSF (phenylmethylsulfonyl fluoride; Wako 25 Pure Chemicals). On the other hand, an MT18 antibody produced by the MT18 hybridoma prepared in Reference Example 1 was bonded to cyanogen bromide-activated Sepharose 4B (Pharmacia) according to a conventional procedure. This MT18 antibody-conjugated Sepharose 4B 30 was mixed with the above-prepared cell lysate to bind the solubilized IL-6R to the MT18 antibody on Sepharose 4B. Substances non-specifically bonded to the Sepharose 4B were washed off, and the IL-6R bound to Sepharose 4B via the MT18 antibody was used as an immunogen.

35 BALB/c mice were intraperitoneally immunized with the above-prepared immunogen, once a week for 4 weeks.

Next, spleen cells were obtained from the immunized mice, and fused with myeloma cells P3U1 using polyethylene glycol according to a conventional procedure. The fused cells were screened as follows. First, a culture supernatant and 0.01 ml of Protein G Sepharose (Pharmacia) were mixed to adsorb immunoglobulin in the supernatant to the Protein G Sepharose. On the other hand, 10^7 U266 cells internally labeled with ^{35}S -methionine were lysed, and the IL-6R was affinity-purified using the MT18-conjugated Sepharose 4B. Next, the ^{35}S -methionine-labeled IL-6R was immunoprecipitated with the above-prepared Protein G Sepharose on which an immunoglobulin had been bonded, and the precipitate was analyzed by SDS/PAGE. As a result, one hybridoma clone producing antibody which specifically bound to the IL-6R was isolated, and designated PM1. The hybridoma PM1 was deposited with the FRI under the Budapest Treaty as FERM BP-2998, on July 10, 1990.

20 Reference Example 3 Construction of Hybridoma
 AUK12-20, AUK64-7 and
 AUK146-15

As an immunogen, a soluble IL-6R (SR 344) was prepared according to a procedure described by Yasukawa, K. et al., J. Biochem. 108, 673-676, 1990. Namely, a plasmid pECEdhfr 344 containing a cDNA coding for IL-6R wherein the 345th codon from the N-terminus had been replaced by a stop codon was transfected to CHO (5E27) cells, the transfected cells were cultured in a serum-free medium (SF-O medium, Sanko Junyaku), and a resulting supernatant was concentrated with an HF-Lab1 system (Tosoh), and purified by Blue-5PW column and Phenyl-5PW column. The purified soluble IL-6R showed a single band in an SDS-PAGE.

35 A female BALB/cAnNCrj mouse (Nippon CREA) was subcutaneously injected with 10 $\mu\text{g}/\text{mouse}$ of the immunogen

in Freund's complete adjuvant (Bacto Adjuvant Complete H 37 Ra, Difco), followed by the second and third injections of the same amount of the immunogen in Freund's incomplete adjuvant (Bacto Adjuvant Incomplete Freund, Difco) two and three weeks after the first injection, respectively. A final immunization (the fourth injection) was carried out without adjuvant into a tail vein one week after the third injection. A serum sample was prepared from the immunized mice, serially 5 diluted with a dilution buffer, and assayed by ELISA according to a procedure described by Goldsmith, P.K., Analytical Biochemistry, 117, 53-60, 1981. Namely, an 10 SR344 (0.1 μ /ml)-coated plate was blocked with 1% BSA, and the diluted sample was added thereon. Mouse IgG 15 bound to the SR344 was measured using goat anti-mouse IgG/alkaline phosphatase (A/P) (ZYMED) and a substrate for alkaline phosphatase (Sigma-104).

After confirming an increase of the anti-SR344 antibody in the serum, spleen cells were obtained from 20 5 BALB/c mice three days after the final immunization. The spleen cells and myeloma cells (P3U1) were mixed at a ratio of 25 : 1, fused using PEG1500, and cultured in 2000 wells at a cell concentration of 0.7 to 25 1.1×10^6 cells/well. Supernatants from the wells were screened for their ability to bind SR344 (the first screening designated as R344 recognition assay), and for 30 their ability to inhibit a binding of SR344 with an interleukin-6 by a IL-6/sIL-6R binding inhibition assay (RBIA). The first screening provided 240 positive wells, and the second screening provided 36 positive wells.

The above-mentioned R344 recognition assay was carried out as follows. Goat anti-mouse Ig (Cappel) 35 (1 μ g/ml)-coated plate (MaxiSorp, Nunc) was blocked with 1% BSA, and 100 μ l/well of hybridoma culture supernatant was added thereon, followed by an incubation at room temperature for one hour. After washing the plate, 20

5 $\mu\text{g}/\text{ml}$ of SR344 was added to each well, and incubation was carried out at room temperature for one hour. The amount of SR344 captured by the immobilized antibody derived from the supernatant was determined by addition of rabbit anti-SR344 IgG (#2, 5 $\mu\text{g}/\text{ml}$), goat anti-rabbit IgG-alkaline phosphatase (A/P) (1:3000, Tago), and of a substrate (1 mg/ml , Sigma-104), followed by measurement of the optical density at 405-600 nm.

10 The above-mentioned RBIA was carried out as follows. MT18 antibody-coated plate was filled with 100 $\mu\text{g}/\text{ml}$ of SR344 (100 $\mu\text{l}/\text{well}$), and incubation was carried out at a room temperature for one hour. After washing the plate, 50 $\mu\text{l}/\text{well}$ of hybridoma supernatant and 50 $\mu\text{g}/\text{well}$ of biotin-interleukin-6 conjugate (20 $\mu\text{g}/\text{ml}$) were

15 simultaneously added to each well, and the wells were incubated at room temperature for one hour. An amount of biotin-IL-6 bound to SR344 was measured by an addition of streptavidin-A/P (1 : 7000, PIERCE) and a corresponding substrate (Sigma-104), followed by a measurement of the

20 optical density at 405-600 nm.

Finally, positive clones were purified by a twice-repeated limiting dilution method, and three hybridoma clones, i.e., AUK12-20, AUK145-15 and AUK64-7, which inhibit the binding of SR344 with the IL-6; and a

25 hybridoma clone AUK181-6, which does not inhibit the binding of SR344 with the IL-6, were obtained.

Industrial Applicability

The present invention provides a reshaped human antibody to the human IL-6R, comprising a human antibody wherein the CDRs of the human V regions are replaced with the CDRs of a mouse monoclonal antibody to the human IL-6R. Since major portion of the reshaped human antibody is derived from a human antibody and the mouse CDRs which are less antigenic, the present reshaped human antibody is less immunogenic to human, and therefore is promised for therapeutic uses.

Reference to Deposited Microorganisms under Rule 13-2 of Budapest Treaty

10	<u>E. Coli</u> DH5 α , pPM-h1	NCIMB 40362	Feb. 12, 1991
	<u>E. Coli</u> DH5 α , p12-h2	NCIMB 40363	Feb. 12, 1991
	<u>E. Coli</u> DH5 α , p64-h2	NCIMB 40364	Feb. 12, 1991
	<u>E. Coli</u> DH5 α , p146-h1	NCIMB 40365	Feb. 12, 1991
	<u>E. Coli</u> DH5 α , pPM-k3	MCIMB 40366	Feb. 12, 1991
15	<u>E. Coli</u> DH5 α , p12-k2	NCIMB 40367	Feb. 12, 1991
	<u>E. Coli</u> DH5 α , p64-k4	NCIMB 40368	Feb. 12, 1991
	<u>E. Coli</u> DH5 α , p146-k3	NCIMB 40369	Feb. 12, 1991

Identification of Deposition No. Deposition Date
25 Microorganism

MT 18	FERM BP-2999	July 10, 1990
PM 1	FERM BP-2998	July 10, 1990

Sequence Listing

SEQ. ID NO : 1

SEQUENCE LENGTH : 40

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGAACTTGC CTGTTAGGCT GTTGGTGCTG

40

SEQ. ID NO : 2

SEQUENCE LENGTH : 39

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGGAGWCAG ACACACTCCT GYTATGGGT

39

SEQ. ID NO : 3

SEQUENCE LENGTH : 40

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGACTGTGC TCACTCAGGT CCTGGSGTTG

40

SEQ. ID NO : 4

SEQUENCE LENGTH : 43

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGAGGRCCC CTGCTCAGWT TYTTGGMWTC TTG

43

SEQ. ID NO : 5

SEQUENCE LENGTH : 40

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGGATTWC AGGTGCAGAT TWTCAGCTTC

40

SEQ. ID NO : 6

SEQUENCE LENGTH : 37

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGAGGCKCY YTGYTSAGYT YCTGRGG

37

SEQ. ID NO : 7

SEQUENCE LENGTH : 41

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGGCCWTCA AGATGGACTC ACAKWYYCWG G

41

SEQ. ID NO : 8

SEQUENCE LENGTH : 41

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGTGGGGAY CTKTTTYCMM TTTTTCAATT G

41

SEQ. ID NO : 9

SEQUENCE LENGTH : 35

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGGTRTCCW CASCTCAGTT CCTTG

35

SEQ. ID NO : 10

SEQUENCE LENGTH : 37

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGTATATAT GTTTGTTGTC TATTCT

37

SEQ. ID NO : 11

SEQUENCE LENGTH : 38

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGGAAGCCC CAGCTCAGCT TCTCTTCC

38

SEQ. ID NO : 12

SEQUENCE LENGTH : 27

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GGATCCCGGG TCGATGGTGG GAAGATG

27

SEQ. ID NO : 13

SEQUENCE LENGTH : 37

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGAAATGCA GCTGGGTCA T STTCTTC

37

SEQ. ID NO : 14

SEQUENCE LENGTH : 36

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGGGATGGA GCTRTATCAT SYTCTT

36

SEQ. ID NO : 15

SEQUENCE LENGTH : 37

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGAAGWTGT GGTTAAACTG GGTTTT

37

SEQ. ID NO : 16

SEQUENCE LENGTH : 35

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGRACCTTG GGYTCAGCTT GRTTT

35

SEQ. ID NO : 17

SEQUENCE LENGTH : 40

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGGACTCCA GGCTCAATT AGTTTCCTT

40

SEQ. ID NO : 18

SEQUENCE LENGTH : 37

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGGCTGTCY TRGSGCTRCT CTTCTGC

37

SEQ. ID NO : 19

SEQUENCE LENGTH : 36

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGGRATGGA GCKGGRTCTT TMTCTT

36

SEQ. ID NO : 20

SEQUENCE LENGTH : 33

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGAGAGTGC TGATTCTTT GTG

33

SEQ. ID NO : 21

SEQUENCE LENGTH : 40

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGGMTTGGG TGTGGAMCTT GCTATTCCCTG

40

SEQ. ID NO : 22

SEQUENCE LENGTH : 37

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACTAGTCGAC ATGGGCAGAC TTACATTCTC ATTCCCTG

37

SEQ. ID NO : 23

SEQUENCE LENGTH : 28

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GGATCCCCGGG CCAGTGGATA GACAGATG

28

SEQ. ID NO : 24

SEQUENCE LENGTH : 393

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : cDNA

ORIGINAL SOURCE

ORGANISM : Mouse

IMADIATE SOURCE

CLONE : p12-k2

FEATURE : 1..60 sig peptide

61..393 mat peptide

SEQUENCE

ATG	GAG	TCA	GAC	ACA	CTC	CTG	CTA	TGG	GTA	CTG	CTG	CTG	CTC	TGG	GTT	CCA	48
Met	Glu	Ser	Asp	Thr	Leu	Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro		
-20				-15						-10					-5		
GGT	TCC	ACT	GGT	GAC	ATT	GTG	CTG	ACA	CAG	TCT	CCT	GCT	TCC	TTA	GGT	96	
Gly	Ser	Thr	Gly	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Gly		
					1				5					10			
GTA	TCT	CTG	GGG	CAG	AGG	GCC	ACC	ATC	TCA	TGC	AGG	GCC	ACC	AAA	AGT	144	
Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Lys	Ser		
					15			20			25						
GTC	AGT	ACA	TCT	GGC	TAT	AGT	TAT	ATG	CAC	TGG	TAC	CAA	CAG	AAA	CCA	192	
Val	Ser	Thr	Ser	Gly	Tyr	Ser	Tyr	Met	His	Trp	Tyr	Gln	Gln	Lys	Pro		
					30			35			40						
GGA	CAG	ACA	CCC	AAA	CTC	CTC	ATC	TAT	CTT	GCA	TCC	AAC	CTA	GAA	TCT	240	
Gly	Gln	Thr	Pro	Lys	Leu	Leu	Ile	Tyr	Leu	Ala	Ser	Asn	Leu	Glu	Ser		
					45			50			55			60			
GGG	GTC	CCT	GCC	AGG	TTC	AGT	GGC	AGT	GGG	TCT	GGG	ACA	GAC	TTC	ACC	288	
Gly	Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr		
					65			70			75						
CTC	AAC	ATC	CAT	CCT	GTG	GAG	GAG	GAG	GAT	GCT	GCA	ACC	TAT	TAC	TGT	336	
Leu	Asn	Ile	His	Pro	Val	Glu	Glu	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys		
					80			85			90						

CAG CAC AGT AGG GAC AAT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG 384
Gln His Ser Arg Glu Asn Pro Tyr Thr Phe Gly Gly Thr Lys Leu
95 100 105
GAA ATA AAA 393
Glu Ile Lys
110

SEQ. ID NO : 25 25

SEQUENCE LENGTH : 405

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : cDNA

ORIGINAL SOURCE

ORGANISM : Mouse

IMADIATE SOURCE

CLONE : p12-h2

FEATURE : 1..57 sig peptide

58..405 mat peptide

SEQUENCE

ATG GGA TGG AGC GGG ATC TTT CTC TTC CTT CTG TCA GGA ACT GCA GGT 48
Met Gly Trp Ser Gly Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
-15 -10 -5

GTC CAC TCT GAG ATC CAG CTG CAG CAG TCT GGA CCT GAG CTG ATG AAG 96
Val His Ser Glu Ile Gln Leu Gln Gln Ser Gly Pro Glu Leu Met Lys
-1 5 10

CCT	GGG	GCT	TCA	GTG	AAG	ATA	TCC	TGC	AAG	GCT	TCT	GGT	TAC	TCA	TTC	144
Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	
15				20						25						
ACT	AGC	TAT	TAC	ATA	CAC	TGG	GTG	AAG	CAG	AGC	CAT	GGA	AAG	ACC	CTT	192
Thr	Ser	Tyr	Tyr	Ile	His	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	Leu	
30				35					40			45				
GAG	TGG	ATT	GGA	TAT	ATT	GAT	CCT	TTC	AAT	GGT	GGT	ACT	AGC	TAC	AAC	240
Glu	Trp	Ile	Gly	Tyr	Ile	Asp	Pro	Phe	Asn	Gly	Gly	Thr	Ser	Tyr	Asn	
50				55						60						
CAG	AAA	TTC	AAG	GGC	AAG	GCC	ACA	TTG	ACT	GTT	GAC	AAA	TCT	TCC	AGC	288
Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	
65				70						75						
ACA	GCC	TAC	ATG	CAT	CTC	AGC	AGC	CTG	ACA	TCT	GAG	GAC	TCT	GCA	GTC	336
Thr	Ala	Tyr	Met	His	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	
80				85						90						
TAT	TAC	TGT	GCA	AGC	GGG	GGT	AAC	CGC	TTT	GCT	TAC	TGG	GGC	CAA	GGG	384
Tyr	Tyr	Cys	Ala	Arg	Gly	Gly	Asn	Arg	Phe	Ala	Tyr	Trp	Gly	Gln	Gly	
95				100						105						
ACT	CTG	GTC	ACT	GTC	TCT	GCA										405
Thr	Leu	Val	Thr	Val	Ser	Ala										
110				115												

SEQ. ID NO : 26

SEQUENCE LENGTH : 381

SEQUENCE TYPE : Nucleic acid

STRANNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : cDNA

ORIGINAL SOURCE

ORGANISM : Mouse

IMMEDIATE SOURCE

CLONE : pPM-k3

FEATURE : 1..60 sig peptide

61..381 mat peptide

SEQUENCE

ATG	GTG	TCC	TCA	GCT	CAG	TTC	CTT	GGT	CTC	CTG	TTG	CTC	TGT	TTT	CAA	48
Met	Val	Ser	Ser	Ala	Gln	Phe	Leu	Gly	Leu	Leu	Leu	Cys	Phe	Gln		
-20															-5	
GGT	ACC	AGA	TGT	GAT	ATC	CAG	ATG	ACA	CAG	ACT	ACA	TCC	TCC	CTG	TCT	96
Gly	Thr	Arg	Cys	Asp	Ile	Gln	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu	Ser	
1								5						10		
GCC	TCT	CTG	GGA	GAC	AGA	GTC	ACC	ATC	AGT	TGC	AGG	GCA	AGT	CAG	GAC	144
Ala	Ser	Leu	Gly	Asp	Arg	Val	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Gln	Asp	
15								20						25		
ATT	AGC	ACT	TAT	TTA	AAC	TGG	TAT	CAG	CAG	AAA	CCA	CAT	CGA	ACT	ATT	192
Ile	Ser	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr	Ile	
30								35						40		
AAA	CTC	CTG	ATC	TAC	TAC	ACA	TCA	AGA	TTA	CAC	TCA	CGA	GTC	CCA	TCA	240
Lys	Leu	Leu	Ile	Tyr	Tyr	Thr	Ser	Arg	Leu	His	Ser	Gly	Val	Pro	Ser	
45								50						55		
AGG	TTC	ACT	GGC	ACT	GGG	TCT	CGA	ACA	GAT	TAT	TCT	CTC	ACC	ATT	AAC	288
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile	Asn	
65								70						75		

AAC CTG GAG CAA GAA GAC ATT GCC ACT TAC TTT TGC CAA CAG GGT AAC	336	
Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn		
80	85	90
ACG CTT CCG TAC ACG TTC GGA CGG GGG ACC AAG CTG GAA ATA AAT	381	
Thr Leu Pro Tyr Thr Phe Gly Gly Thr Lys Leu Glu Ile Asn		
95	100	105

SEQ. ID NO : 27

SEQUENCE LENGTH : 411

SEQUENCE TYPE : Nucleic acid

STRANDEDNESS : Double

TOPOLOGY : Linear

STRANDNESS : Double

MOLECULE TYPE : cDNA

ORIGINAL SOURCE

ORGANISM : Mouse

IMADIATE SOURCE

CLONE : pPM-h1

FEATURE : 1..54 sig peptide

55..411 mat peptide

SEQUENCE

ATG AGA GTG CTG ATT CTT TTG TGG CTG TTC ACA CCC TTT CCT GGT ATC	48
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Met Arg Val Leu Ile Leu Leu Trp Leu Phe Thr Ala Phe Pro Gly Ile

-15 -10 -5

CTG TCT GAT GTG CAG CTT CAG GAG TCG GGA CCT GTC CTG GTG AAG CCT	96
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Leu Ser Asp Val Gln Leu Gln Glu Ser Gly Pro Val Leu Val Lys Pro

-1 5 10

TCT	CAG	TCT	CTG	TCC	CTC	ACC	TGC	ACT	GTC	ACT	GGC	TAC	TCA	ATC	ACC	144
Ser	Gln	Ser	Leu	Ser	Leu	Thr	Cys	Thr	Val	Thr	Gly	Tyr	Ser	Ile	Thr	
15		20					25						30			
ACT	GAT	CAT	GCC	TGG	AGC	TGG	ATC	CGG	CAG	TTT	CCA	GGA	AAC	AAA	CTG	192
Ser	Asp	His	Ala	Trp	Ser	Trp	Ile	Arg	Gln	Phe	Pro	Gly	Asn	Lys	Leu	
35							40						45			
GAG	TGG	ATG	GGC	TAC	ATA	AGT	TAC	AGT	GGT	ATC	ACT	ACC	TAC	AAC	CCA	240
Glu	Trp	Met	Gly	Tyr	Ile	Ser	Tyr	Ser	Gly	Ile	Thr	Thr	Tyr	Asn	Pro	
50							55						60			
TCT	CTC	AAA	AGT	CGA	ATC	TCT	ATC	ACT	CGA	GAC	ACA	TCC	AAG	AAC	CAG	288
Ser	Leu	Lys	Ser	Arg	Ile	Ser	Ile	Thr	Arg	Asp	Thr	Ser	Lys	Asn	Gln	
65							70						75			
TTC	TTC	CTA	CAG	TTG	AAT	TCT	GTG	ACT	ACT	GGG	GAC	ACG	TCC	ACA	TAT	336
Phe	Phe	Leu	Gln	Leu	Asn	Ser	Val	Thr	Thr	Gly	Asp	Thr	Ser	Thr	Tyr	
80							85						90			
TAC	TGT	GCA	AGA	TCC	CTA	GCT	CGG	ACT	ACG	GCT	ATG	GAC	TAC	TGG	GGT	384
Tyr	Cys	Ala	Arg	Ser	Leu	Ala	Arg	Thr	Thr	Ala	Met	Asp	Tyr	Trp	Gly	
95							100						105			110
CAA	GGA	ACC	TCA	GTC	ACC	GTC	TCC	TCA								411
Gln	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser								
																115

SEQ. ID NO : 28

SEQUENCE LENGTH : 393

SEQUENCE TYPE : Nucleic acid

STRANNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : cDNA

ORIGINAL SOURCE

ORGANISM : Mouse

IMMEDIATE SOURCE

CLONE : p64-k4

FEATURE : 1..60 sig peptide

61..393 mat peptide

SEQUENCE

ATG	GAG	TCA	GAC	ACA	CTC	CTG	CTA	TGG	GTG	CTG	CTG	CTC	TGG	GTT	CCA	48
Met	Glu	Ser	Asp	Thr	Leu	Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro	
-20				-15					-10					-5		
GGT	TCC	ACA	GGT	GAC	ATT	GTG	TTG	ATC	CAA	TCT	CCA	GCT	TCT	TTG	GCT	96
Gly	Ser	Thr	Gly	Asp	Ile	Val	Leu	Ile	Gln	Ser	Pro	Ala	Ser	Leu	Ala	
				-1					5				10			
GTG	TCT	CTA	GGG	CAG	AGG	GCC	ACC	ATA	TCC	TGC	AGA	GCC	ACT	GAA	AGT	144
Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Glu	Ser	
				15				20				25				
GTT	GAT	AGT	TAT	GGC	AAT	AGT	TTT	ATG	CAC	TGG	TAC	CAG	CAG	AAA	CCA	192
Val	Asp	Ser	Tyr	Gly	Asn	Ser	Phe	Met	His	Trp	Tyr	Gln	Gln	Lys	Pro	
				30				35				40				
GGA	CAG	CCA	CCC	AAA	CTC	CTC	ATC	TAT	CGT	GCA	TCC	AAC	CTA	GAA	TCT	240
Gly	Gln	Pro	Pro	Lys	Leu	Leu	Ile	Tyr	Arg	Ala	Ser	Asn	Leu	Glu	Ser	
				45				50				55			60	
GGG	ATC	CCT	GCC	AGG	TTC	AGT	GGC	AGT	GGG	TCT	AGG	ACA	GAC	TTC	ACC	288
Gly	Ile	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Arg	Thr	Asp	Phe	Thr	
				65				70				75				

CTC ACC ATT AAT CCT GTG GAG CCT GAT GAT GTT GCA ACC TAT TAC TGT	336	
Leu Thr Ile Asn Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys		
80	85	90
CAG CAA ACT AAT GAG GAT CCT CCC ACG TTC GGT GCT GGG ACC AAG CTG	384	
Gln Gln Ser Asn Glu Asp Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu		
95	100	105
GAG CTG AAA	393	
Glu Leu Lys		
110		

SEQ. ID NO : 29

SEQUENCE LENGTH : 417

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : cDNA

ORIGINAL SOURCE

ORGANISM : Mouse

IMADIATE SOURCE

CLONE : p64-h2

FEATURE : 1..57 sig peptide

58..417 mat peptide

SEQUENCE

ATG CGA TGG AGC GCG GTC TTT ATC TTC CTC CTG TCA GTA ACT GCA GGT	48
Met Gly Trp Ser Gly Val Phe Ile Phe Leu Leu Ser Val Thr Ala Gly	

GTC CAC TCC CAG GTT CAA TTG CAG CAG TCT GCA GCT GAG TTG ATG AAG	96		
Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys			
-1	5	10	
CCT CGG CCC TCA GTG AAG ATC TCC TGC AAG GCT ACT GGC TAC ACA TTC	144		
Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe			
15	20	25	
AGT AGT TAT TGG ATA GTG TGG ATA AAG CAG AGG CCT GGA CAT GGC CTT	192		
Ser Ser Tyr Trp Ile Val Trp Ile Lys Gln Arg Pro Gly His Gly Leu			
30	35	40	45
GAG TGG ATT GGA GAG ATT TTA CCT GGA ACC GGT AGT ACT AAC TAC AAT	240		
Glu Trp Ile Gly Glu Ile Leu Pro Gly Thr Gly Ser Thr Asn Tyr Asn			
50	55	60	
GAG AAA TTC AAG GGC AAG GCC ACA TTC ACT GCA GAT ACA TCT TCC AAC	288		
Glu Lys Phe Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn			
65	70	75	
ACA GCC TAC ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCC GTC	336		
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val			
80	85	90	
TAT TAC TGT GCA ACT CTA GAC AGC TCG GGC TAC TAT GCT ATG GAC TAT	384		
Tyr Tyr Cys Ala Ser Leu Asp Ser Ser Gly Tyr Tyr Ala Met Asp Tyr			
95	100	105	
TGG CGT CAA GGA ACC TCA GTC ACC GTC TCC TCA	417		
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser			
110	115	120	

SEQ. ID NO : 30

SEQUENCE LENGTH : 381

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : cDNA

ORIGINAL SOURCE

ORGANISM : Mouse

IMADIATE SOURCE

CLONE : p146-k3

FEATURE : 1..60 sig peptide

61..381 mat peptide

SEQUENCE

ATG	GTG	TCC	ACA	CCT	CAG	TTC	CTT	GGT	CTC	CTG	TTG	ATC	TGT	TTT	CAA	48
Met	Val	Ser	Thr	Pro	Gln	Phe	Leu	Gly	Leu	Leu	Leu	Ile	Cys	Phe	Gln	
-20						-15						-10			-5	
GGT	ACC	AGA	TGT	GAT	ATC	CAG	ATG	ACA	CAG	ACT	ACA	TCC	TCC	CTG	TCT	96
Gly	Thr	Arg	Cys	Asp	Ile	Gln	Met	Thr	Gln	Thr	Thr	Ser	Ser	Leu	Ser	
-1							5					10				
GCC	TCT	CTG	GGA	GAC	AGA	GTC	ACC	ATC	AGT	TGC	AGG	GCA	AGT	CAG	GAC	144
Ala	Ser	Leu	Gly	Asp	Arg	Val	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Gln	Asp	
15							20					25				
ATT	AGT	AAT	TAT	TTA	AAC	TGG	TAT	CAA	CAG	AAA	CCA	GAT	GGA	ACT	GTT	192
Ile	Ser	Asn	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Asp	Gly	Thr	Val	
30							35				40					
AAA	CTC	CTG	ATC	TAC	TAT	ACA	TCA	AGA	TTA	CAC	TCA	GGA	GTC	CCA	TCA	240
Lys	Leu	Leu	Ile	Tyr	Tyr	Thr	Ser	Arg	Leu	His	Ser	Gly	Val	Pro	Ser	
45							50				55			60		

AGG	TTC	AGT	GGC	AGT	GGG	TCT	GGA	ACA	GAT	TAT	TCT	CTC	ACC	ATT	AGC	288
Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Tyr	Ser	Leu	Thr	Ile	Ser	
	65							70						75		
AAC	CTG	GAG	CAA	GAA	GAT	ATT	GCC	AGT	TAC	TTT	TGC	CAA	CAG	GGT	TAT	336
Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala	Ser	Tyr	Phe	Cys	Gln	Gln	Gly	Tyr	
	80							85					90			
ACG	CCT	CCC	TGG	ACG	TTC	GGT	GGA	GCC	ACC	AAG	TTC	CAA	ATC	AAA	381	
Thr	Pro	Pro	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys		
	95							100					105			

SEQ. ID NO : 31

SEQUENCE LENGTH : 402

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : cDNA

ORIGINAL SOURCE

ORGANISM : Mouse

IMADIATE SOURCE

CLONE : p146-h1

FEATURE : 1..51 sig peptide

52..402 mat peptide

SEQUENCE

ATG	GAG	CTG	GAT	CTT	TAT	CTT	ATT	CTG	TCA	GTA	ACT	TCA	GGT	GTC	TAC	48
Met	Glu	Leu	Asp	Leu	Tyr	Leu	Ile	Leu	Ser	Val	Thr	Ser	Gly	Val	Tyr	

TCA CAG GTT CAG CTC CAG CAG TCT GGG GCT GAG CTG GCA AGA CCT GGG	96		
Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly			
-1	5	10	15
GCT TCA GTG AAG TTG TCC TGC AAG GCT TCT GCC TAC ACC TTT ACT AAC	144		
Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn			
20	25	30	
TAC TGG GTG CAG TGG GTA AAA CAG AGG CCT GCA CAG CGT CTG GAA TGG	192		
Tyr Trp Val Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp			
35	40	45	
ATT GGG TCT ATT TAT CCT GGA GAT GGT GAT ACT AGG AAC ACT CAG AAG	240		
Ile Gly Ser Ile Tyr Pro Gly Asp Gly Asp Thr Arg Asn Thr Gln Lys			
50	55	60	
TTC AAG GGC AAG GCC ACA TTG ACT GCA GAT AAA TCC TCC ATC ACA GCC	288		
Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ile Thr Ala			
65	70	75	
TAC ATG CAA CTC ACC AGC TTG GCA TCT GAG GAC TCT GCG GTC TAT TAC	336		
Tyr Met Gln Leu Thr Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Tyr			
80	85	90	95
TGT GCA AGA TCG ACT GGT AAC CAC TTT GAC TAC TGG GCC CAA GCC ACC	384		
Cys Ala Arg Ser Thr Gly Asn His Phe Asp Tyr Trp Gly Gln Gly Thr			
100	105	110	
ACT CTC ACA GTC TCC TCA	402		
Thr Leu Thr Val Ser Ser			

SEQ. ID NO : 32

SEQUENCE LENGTH : 35

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACAAAGCTTC CACCATGGAG TCAGACACAC TCCTG

35

SEQ. ID NO : 33

SEQUENCE LENGTH : 36

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GGCTAAGCTT CCACCATGGG ATGGACCGGG ATCTTT

36

SEQ. ID NO : 34

SEQUENCE LENGTH : 35

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

CTTGGATCCA CTCACGTTTT ATTTCCAGCT TGGTC

35

SEQ. ID NO : 35

SEQUENCE LENGTH : 36

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GTTGGATCCA CTCACCTGCA GAGACAGTTA CCAGAG

36

SEQ. ID NO : 36

SEQUENCE LENGTH : 35

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

CTTGGATCCA CTCACGATT ATTCCAGCT TGGTC

35

SEQ. ID NO : 37

SEQUENCE LENGTH : 35

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

CTTGGATCCA CTCACGTTT ATTCCAGCT TGGTC

35

SEQ. ID NO : 38

SEQUENCE LENGTH : 36

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACAAAGCTTC CACCATGGTG TCCTCAGCTC AGTTCC

36

SEQ. ID NO : 39

SEQUENCE LENGTH : 39

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

TGTTAGATCT ACTCACCTGA GGAGACACTG ACTGAGGTT

39

SEQ. ID NO : 40

SEQUENCE LENGTH : 36

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GTCTAACGTT CCACCATGAG AGTGCTGATT CTTTG

36

SEQ. ID NO : 41

SEQUENCE LENGTH : 17

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

TACGCAAACC GCCTCTC

17

SEQ. ID NO : 42

SEQUENCE LENGTH : 18

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GACTGCACCA TATGCGGT

18

SEQ. ID NO : 43

SEQUENCE LENGTH : 55

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACCGTGTCTG GCTACACCTT CACCAGCCAT CATGCCTGGA GCTGGGTGAG ACAGC

55

SEQ. ID NO : 44

SEQUENCE LENGTH : 63

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

TGACTGGATT GGATACATTA GTTATAGTGG AATCACAACC TATAATCCAT	50
CTCTCAAATC CAG	63

SEQ. ID NO : 45

SEQUENCE LENGTH : 54

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

TATTATTGTG CAAGATCCCT AGCTCGGACT ACGGCTATGG ACTACTGGGG TCAA	54
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SEQ. ID NO : 46

SEQUENCE LENGTH : 27

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GTGACAATGC TGAGAGACAC CAGCAAG	27
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SEQ. ID NO : 47

SEQUENCE LENGTH : 24

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GGTGTCCACT CCGATGTCCA ACTG

24

SEQ. ID NO : 48

SEQUENCE LENGTH : 27

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GGTCTTGACT GGATGGATA CATTAGT

27

SEQ. ID NO : 49

SEQUENCE LENGTH : 29

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GTGTCTGGCT ACTCAATTAC CACCATCAT

29

SEQ. ID NO : 50

SEQUENCE LENGTH : 48

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

TGTAGAGCCA GCCAGGACAT CACCAAGTTAC CTGAACTGGT ACCAGCAG

48

SEQ. ID NO : 51

SEQUENCE LENGTH : 42

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ATCTACTACA CCTCCAGACT GCACTCTGGT GTGCCAAGCA GA

42

SEQ. ID NO : 52

SEQUENCE LENGTH : 50

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACCTACTACT GCCAACAGGG TAACACGCTT CCATACACGT TCGGCCAAGG

50

SEQ. ID NO : 53

SEQUENCE LENGTH : 27

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

AGCGGTACCG ACTACACCTT CACCATC

27

SEQ. ID NO : 54

SEQUENCE LENGTH : 706

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

ORIGINAL SOURCE

ORGANISM : Mouse and Human

IMADIATE SOURCE

CLONE : pUC-RVh-PM1f

FEATURE : gene coding for H chain V region version (f) of reshaped
human PM-1 antibody to human IL-6R

amino acid -20--1 : leader

amino acid 1 - 30 : FR1

amino acid 31 - 36 : CDR1

amino acid 37 - 50 : FR2

amino acid 51 - 66 : CDR2

amino acid 67 - 98 : FR3

amino acid 99-108:CDR3

amino acid 109-119:FR4

nucleotide 1-6 Hind III site

nucleotide 54-135 intron

nucleotide 258-348 intron/aberrant splicing

nucleotide 505-706 intron

nucleotide 701-706 Bam HI site

SEQUENCE

AAGCTTC ATG GCA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT 49

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala

-15 -10

ACA G GTAACGGGCT CACAGTAGCA GGCTTGAGGT CTGGACATAT ATATGGTCA 103

Thr

-5

CAATGACATC CACTTGCCT TTCTCTCCAC AG GT GTC CAC TCC CAG GTC CAA 155

Gly Val His Ser Gln Val Gln

1

CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG ACC CTG AGC 203

Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln Thr Leu Ser

5 10 15

CTG ACC TGC ACC GTG TCT GGC TAC TCA ATT ACC AGC GAT CAT GCC TGG 251

Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr Ser Asp His Ala Trp

20 25 30 35

AGC TGG GTG AGA CAG CCA CCT GGA CGA GGT CTT GAG TGG ATT GGA TAC 299

Ser Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile Gly Tyr

40 45 50

ATT ACT TAT AGT GGA ATC ACA ACC TAT AAT CCA TCT CTC AAA TCC AGA	347		
Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu Lys Ser Arg			
55	60	65	
GTG ACA ATG CTG AGA GAC ACC AGC AAG AAC CAG TTC AGC CTG AGA CTC	395		
Val Thr Met Leu Arg Asp Thr Ser Lys Asn Gln Phe Ser Leu Arg Leu			
70	75	80	
AGC AGC GTG ACA CCC GCC GAC ACC GCG GTT TAT TAT TGT GCA AGA TCC	443		
Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser			
85	90	95	
CTA GCT CGG ACT ACG GCT ATG GAC TAC TGG GGT CAA GGC AGC CTC GTC	491		
Leu Ala Arg Thr Thr Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val			
100	105	110	115
ACA GTC TCC TCA G GTGAGTCCTT ACAACCTCTC TCTTCTATT AGCTTAAATA	544		
Thr Val Ser Ser			
GATTTTACTG CATTGTTGG GGGGGAAATG TGTGTATCTG AATTCAGGT CATGAAGGAC	604		
TAGGGACACC TTGGGACTCA GAAAGGGTCA TTGGGAGCCC GGGCTGATGC AGACAGACAT	664		
CCTCAGCTCC CAGACTTCAT GCCCAGAGAT TTATAGGGAT CC	706		

SEQ. ID NO : 55

SEQUENCE LENGTH : 506

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

ORIGINAL SOURCE

ORGANISM : Mouse and Human

IMADIASTE SOURCE

CLONE : pUC-RV1-PM1a

FEATURE : gene coding for L chain V region version (a) of reshaped
human PM-1 antibody to human IL-6R

amino acid -20--1 : leader

amino acid 1 - 23 : FR1

amino acid 24 - 34 : CDR1

amino acid 35 - 49 : FR2

amino acid 50 - 56 : CDR2

amino acid 57 - 88 : FR3

amino acid 89 - 97 : CDR3

amino acid 98 - 117 : FR4

nucleotide 1 - 6 : Hind III site

nucleotide 54 - 135 : intron

nucleotide 268 - 376 : intron/aberrant splicing

nucleotide 469 - 506 : intron

nucleotide 501 - 506 : Bam HI site

SEQUENCE

AAGCTTC ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTC GCA ACA GCT 49

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala

-15

-10

ACA G CTAAGGGCT CACAGTACCA GGCTTGAGGT CTGGACATAT ATATGGGTGA 103

Thr

-5

CAATGACATC CACTTGCCT TTCTCTCCAC AG GT GTC CAC TCC GAC ATC CAG 155

Gly Val His Ser Asp Ile Gin

ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC ACC GTG GGT GAC AGA GTG	203
Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val	
5 10 15	
ACC ATC ACC TGT AGA GCC ACC CAG GAC ATC AGC AGT TAC CTG AAT TGG	251
Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Ser Tyr Leu Asn Trp	
20 25 30 35	
TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG CTG CTG ATC TAC TAC ACC	299
Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Thr	
40 45 50	
TCC AGA CTG CAC TCT GGT GTG CCA AGC AGA TTC AGC GGT AGC GGT AGC	347
Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser	
55 60 65	
GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC CTC CAG CCA GAG GAC ATC	395
Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile	
70 75 80	
GCT ACC TAC TAC TGC CAA CAG GGT AAC ACG CTT CCA TAC ACG TTC GGC	443
Ala Thr Tyr Tyr Cys Gln Gln Gly Asn Thr Leu Pro Tyr Thr Phe Gly	
85 90 95	
CAA GGG ACC AAG CTG GAA ATC AAA C GTGAGTAGAA TTTAAACTTT	488
Gln Gly Thr Lys Val Glu Ile Lys	
100 105	
GCTTCCTCAG TTGGATCC	506

SEQ. ID NO : 56

SEQUENCE LENGTH : 438

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

ORIGINAL SOURCE

ORGANISM : Mouse and Human

IMADIATE SOURCE

CLONE : pUC-RVh-PM1f-4

FEATURE : gene, excluding introns, coding for H chain V region

version (f) of reshaped human PM-1 antibody to human IL-6R

amino acid -20--1 : leader

amino acid 1 - 30 : FR1

amino acid 31 - 36 : CDR1

amino acid 37 - 50 : FR2

amino acid 51 - 66 : CDR2

amino acid 67 - 98 : FR3

amino acid 99 - 108: CDR3

amino acid 109 - 119: FR4

nucleotide 1 - 6 : Hind III site

nucleotide 432 - 438: Bam HI site

SEQUENCE

AAGCTTCCAC C ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTC GCA ACA 50

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr

-15

-10

GCT ACA GGT GTC CAC TCC CAG GTC CAA CTG CAG GAC AGC GGT CCA GCT 98

Ala Thr Gly Val His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly

-5

1

5

10

CTT	GTG	AGA	CCT	AGC	CAG	ACC	CTG	AGC	CTG	ACC	TGC	ACC	GTG	TCT	GGC	146
Leu	Val	Arg	Pro	Ser	Gln	Thr	Leu	Ser	Leu	Thr	Cys	Thr	Val	Ser	Gly	
	15						20						25			
TAC	TCA	ATT	ACC	AGC	GAT	CAT	GCC	TGG	AGC	TGG	GTT	CGC	CAG	CCA	CCT	194
Tyr	Ser	Ile	Thr	Ser	Asp	His	Ala	Trp	Ser	Trp	Val	Arg	Gln	Pro	Pro	
	30						35					40				
GGA	CGA	GGT	CTT	GAC	TGG	ATT	GGA	TAC	ATT	AGT	TAT	AGT	GGA	ATC	ACA	242
Gly	Arg	Gly	Leu	Glu	Trp	Ile	Gly	Tyr	Ile	Ser	Tyr	Ser	Gly	Ile	Thr	
	45			50						55						
ACC	TAT	AAT	CCA	TCT	CTC	AAA	TCC	AGA	GTG	ACA	ATG	CTG	AGA	GAC	ACC	290
Thr	Tyr	Asn	Pro	Ser	Leu	Lys	Ser	Arg	Val	Thr	Met	Leu	Arg	Asp	Thr	
	60			65					70							
AGC	AAG	AAC	CAG	TTC	AGC	CTG	AGA	CTC	AGC	AGC	GTG	ACA	GCC	GCC	GAC	338
Ser	Lys	Asn	Gln	Phe	Ser	Leu	Arg	Leu	Ser	Ser	Val	Thr	Ala	Ala	Asp	
	75			80					85			90				
ACC	GCG	GTT	TAT	TAT	TGT	GCA	AGA	TCC	CTA	GCT	CGG	ACT	ACG	GCT	ATG	386
Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Ser	Leu	Ala	Arg	Thr	Thr	Ala	Met	
	95					100						105				
GAC	TAC	TGG	GGT	CAA	GGC	AGC	CTC	GTC	ACA	GTC	TCC	TCA	G	GTGAGTGGAT	436	
Asp	Tyr	Trp	Gly	Gln	Gly	Ser	Leu	Val	Thr	Val	Ser	Ser				
	110					115										
CC														438		

SEQ. ID NO : 57

SEQUENCE LENGTH : 402

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

ORIGINAL SOURCE

ORGANISM : Mouse and Human

IMADIATE SOURCE

CLONE : pUC-RV1-PM1a

FEATURE : gene, excluding introns, coding for L chain V region

version (a) of reshaped human PM-1 antibody to human IL-6R

amino acid - 1 -- 19:leader

amino acid 1 - 23 : FR1

amino acid 24 - 34 : CDR1

amino acid 35 - 49 : FR2

amino acid 50 - 56 : CDR2

amino acid 57 - 88 : FR3

amino acid 89 - 97 : CDR3

amino acid 98 - 107:FR4

nucleotide 1 - 6 : Hind III site

nucleotide 397 - 402: Bam HI site

SEQUENCE

AAGCTTCCAC C ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA CCA ACA 50

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr

-15 -10

GCT ACA GGT GTC CAC TCC GAC ATC CAG ATG ACC CAG AGC CCA AGC AGC 98

Ala Thr Gly Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser

-5

1

5

10

CTG	AGC	GCC	AGC	GTG	GGT	GAC	AGA	GTG	ACC	ATC	ACC	TGT	AGA	GCC	AGC	146
Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	
									15	20	25					
CAG	GAC	ATC	AGC	ACT	TAC	CTG	AAT	TGG	TAC	CAG	CAG	AAG	CCA	CGA	AAG	194
Gln	Asp	Ile	Ser	Ser	Tyr	Leu	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	
									30	35	40					
GCT	CCA	AAG	CTG	CTG	ATC	TAC	TAC	ACC	TCC	AGA	CTG	CAC	TCT	GGT	GTG	242
Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Tyr	Thr	Ser	Arg	Leu	His	Ser	Gly	Val	
									45	50	55					
CCA	AGC	AGA	TTC	AGC	GGT	AGC	GGT	AGC	GGT	ACC	GAC	TTC	ACC	TTC	ACC	290
Pro	Ser	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Phe	Thr	
									60	65	70					
ATC	AGC	AGC	CTC	CAG	CCA	GAG	GAC	ATC	GCT	ACC	TAC	TAC	TGC	CAA	CAG	338
Ile	Ser	Ser	Leu	Gln	Pro	Glu	Asp	Ile	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	
									75	80	85		90			
GGT	AAC	ACG	CTT	CCA	TAC	ACG	TTC	GGC	CAA	GGG	ACC	AAG	GTG	GAA	ATC	386
Gly	Asn	Thr	Leu	Pro	Tyr	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	
									95	100	105					
AAA	C	GTGAGTGGAT	CC												402	
Lys																

SEQ. ID NO : 58

SEQUENCE LENGTH : 36

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

TAAGGATCCA CTCACCTGAG GAGACTGTGA CGAGGC

36

SEQ. ID NO : 59

SEQUENCE LENGTH : 32

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ATCAAGCTTC CACCATGGGA TGGAGCTGTA TC

32

SEQ. ID NO : 60

SEQUENCE LENGTH : 30

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

AATGGATCCA CTCACGTTTC ATTTCCACCT

30

SEQ. ID NO : 61

SEQUENCE LENGTH : 33

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

CATGCCCTGGA GCTGGGTTCG CCAGCCACCT GGA

33

SEQ. ID NO : 62

SEQUENCE LENGTH : 33

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

TCCAGGTGGC TGGCGAACCC AGCTCCAGGC ATG

33

SEQ. ID NO : 63

SEQUENCE LENGTH : 30

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

CAGCAGAAAGC CAGGAAAGGC TCCAAAGCTG

30

SEQ. ID NO : 64

SEQUENCE LENGTH : 30

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

CACCTTGGCA GCCTTCCCTG GCTTCTGCTG

30

SEQ. ID NO : 65

SEQUENCE LENGTH : 66

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACCTCTAGAG CCAGCAAGAG TGTTAGTACA TCTGGCTATA GTTATATGCA

50

CTGGTACCAAG CAGAAG

66

SEQ. ID NO : 66

SEQUENCE LENGTH : 15

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GCTGGCTCTA CAGGT

15

SEQ. ID NO : 67

SEQUENCE LENGTH : 48

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

AAGCTGCTGA TCTACCTTCC ATCCACCCCTG GAATCTGGTG TGCCAACC

48

SEQ. ID NO : 68

SEQUENCE LENGTH : 15

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GTAGATCAGC AGCTT

15

SEQ. ID NO : 69

SEQUENCE LENGTH : 48

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GCTACCTACT ACTGCCAGCA CACTAGGGAG ACCCCATACA CGTTCGCC

48

SEQ. ID NO : 70

SEQUENCE LENGTH : 15

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

CTGGCAGTAG GTAGC

15

SEQ. ID NO : 71

SEQUENCE LENGTH : 414

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

ORIGINAL SOURCE

ORGANISM : Mouse and Human

IMADIATE SOURCE

CLONE : pUC-RV1-1220a

FEATURE : gene, excluding introns, coding for L chain V region version

(a) of reshaped human AUK12-20 antibody to human IL-6R

amino acid -19--1:leader

amino acid 1 - 23 : FR1

amino acid 24 - 38 : CDR1

amino acid 39 - 53 : FR2

amino acid 54 - 60 : CDR2

amino acid 61 - 92 : FR3

amino acid 93 - 101:CDR3

amino acid 102 - 111:FR4

nucleotide 1 - 6 : Hind III site

nucleotide 408 - 414: Bam HI site

SEQUENCE

AAGCTTCCAC C ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA 50
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr
-15 -10
GCT ACA GGT GTC CAC TCC GAC ATC CAG ATG ACC CAG AGC CCA AGC AGC 98
Ala Thr Gly Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
-5 -1 1 5 10
CTG AGC GCC AGC GTG GGT GAC AGA GTG ACC ATC ACC TGT AGA GCC AGC 146
Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
15 20 25
AAG AGT GTT AGT ACA TCT GGC TAT ACT TAT ATG CAC TGG TAC CAG CAG 194
Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln
30 35 40
AAG CCA CGA AAG GCT CCA AAG CTG CTG ATC TAC CTT GCA TCC AAC CTG 242
Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu
45 50 55
GAA TCT GGT GTG CCA AGC AGA TTC AGC GGT AGC GGT ACC ACC GAC 290
Glu Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
60 65 70
TTC ACC TTC ACC ATC ACC AGC CTC CAG CCA GAG GAC ATC GCT ACC TAC 338
Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr
75 80 85 90
TAC TGC CAG CAC ACT AGG GAG AAC CCA TAC ACG TTC GGC CAA GGG ACC 386
Tyr Cys Gln His Ser Arg Glu Asn Pro Tyr Thr Phe Gly Gln Gly Thr
95 100 105

AAG GTG GAA ATC AAA CGTGACTGGA TCC

414

Lys Val Glu Ile Lys

110

SEQ. ID NO : 72

SEQUENCE LENGTH : 45

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GGTTATTCCAT TCACTAGTTA TTACATACAC TGGGTTAGAC AGGCC

45

SEQ. ID NO : 73

SEQUENCE LENGTH : 27

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

AGTGAATGAA TAACCGCTAG CTTTACA

27

SEQ. ID NO : 74

SEQUENCE LENGTH : 69

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GAGTGGGTGG GCTATATTGA TCCTTTCAAT CGTGGTACTA CCTATAATCA 50

GAAGTTCAAG CCCAGGGTT 69

SEQ. ID NO : 75

SEQUENCE LENGTH : 15

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ATAGCCCCACC CACTC 15

SEQ. ID NO : 76

SEQUENCE LENGTH : 36

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GGGGGTAAACC GCTTTGCTTA CTGGGGACAG GGTACC 36

SEQ. ID NO : 77

SEQUENCE LENGTH : 36

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

AGCAAAGCGG TTACCCCTC TGGCGCACTA GTAGAC

36

SEQ. ID NO : 78

SEQUENCE LENGTH : 30

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

CAAGGTTACC ATGACCGTGG ACACCTCTAC

30

SEQ. ID NO : 79

SEQUENCE LENGTH : 30

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

CACGGTCATG GTAACCTTGC CCTTGAACCTT

30

SEQ. ID NO : 80

SEQUENCE LENGTH : 30

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GGGCTCGAAT GGATTGGCTA TATTGATCCT

30

SEQ. ID NO : 81

SEQUENCE LENGTH : 30

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

AGGATCAATA TAGCCAATCC ATT CGAGCCC

30

SEQ. ID NO : 82

SEQUENCE LENGTH : 16

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GTAAAACGAG CCCAGT

16

SEQ. ID NO : 83

SEQUENCE LENGTH : 17

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

AACAGCTATG ACCATGA

17

SEQ. ID NO : 84

SEQUENCE LENGTH : 433

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

ORIGINAL SOURCE

ORGANISM : Mouse and Human

IMADIATE SOURCE

CLONE : pUC-RVh-1220b

FEATURE : gene, excluding intron, coding for H chain V region version

(b) of reshaped human AUK12-20 antibody to human IL-6R

amino acid -19--1:leader

amino acid 1 - 30 : FR1

amino acid 31 - 35 : CDR1

amino acid 36 - 49 : FR2

amino acid 50 - 66 : CDR2

amino acid 67 - 98 : FR3

amino acid 99 - 105:CDR3

amino acid 106 - 116:FR4

nucleotide 1 - 6 : Hind III site

nucleotide 427 - 433: Bam HI site

SEQUENCE

AAGCTTGC_{CC} ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 51
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala
-15 -10

GTG GCT CCT GGG CCC CAC ACC CAG GTG CAA CTA GTG CAG TCC GCC GCC 99
Val Ala Pro Gly Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Ala
-5 -1 1 5

CAA GTG AAG AAA CCC GGT GCT TCC GTG AAA GTC AGC TGT AAA GCT AGC 147
Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser
10 15 20 25

GGT TAT TCA TTC ACT AGT TAT TAC ATA CAC TGG GTT AGA CAG GCC CCA 195
Gly Tyr Ser Phe Thr Ser Tyr Tyr Ile His Trp Val Arg Gln Ala Pro
30 35 40

GCC CAA CGG CTC GAG TGG GTG GCC TAT ATT GAT CCT TTC AAT GGT GGT 243
Gly Gln Gly Leu Glu Trp Val Gly Tyr Ile Asp Pro Phe Asn Gly Gly
45 50 55

ACT AGC TAT AAT CAG AAG TTC AAG GGC AAG GTT ACC ATG ACC GTG GAC 291
Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys Val Thr Met Thr Val Asp
60 65 70

ACC TCT ACA AAC ACC GCC TAC ATG GAA CTG TCC AGC CTG CGC TCC GAG 339
Thr Ser Thr Asn Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu
75 80 85

GAC ACT GCA GTC TAC TAC TGC GCC AGA GGG CGT AAC CGC TTT GCT TAC 387
Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Gly Asn Arg Phe Ala Tyr
90 95 100 105

TGG GGA CAG GGT ACC CTT GTC ACC GTC AGT TCA GGTGAGTGGA TCC 433
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

SEQ. ID NO : 85

SEQUENCE LENGTH : 433

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

ORIGINAL SOURCE

ORGANISM : Mouse and Human

IMADIATE SOURCE

CLONE : pUC-RVh-1220d

FEATURE : gene, excluding intron, coding for H chain V region version

(d) of reshaped human antibody AUK12-20 to human IL-6R

amino acid -19--1:leader

amino acid 1 - 30 : FR1

amino acid 31 - 35 : CDR1

amino acid 36 - 49 : FR2

amino acid 50 - 66 : CDR2

amino acid 67 - 98 : FR3

amino acid 99 - 105:CDR3

amino acid 106 - 116:FR4

nucleotide 1 - 6 : Hind III site

nucleotide 427 - 433: Bam HI site

SEQUENCE

AAGCTTGGCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 51
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala
-15 -10
GTG GCT CCT GGG GCC CAC AGC CAG GTG CAA CTA GTG CAG TCC GCC 99
Val Ala Pro Gly Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Ala
-5 -1 1 5
GAA GTG AAG AAA CCC GGT GCT TCC GTG AAA GTC ACC TGT AAA GCT AGC 147
Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser
10 15 20 25
GGT TAT TCA TTC ACT AGT TAT TAC ATA CAC TGG GTT AGA CAG GCC CCA 195
Gly Tyr Ser Phe Thr Ser Tyr Tyr Ile His Trp Val Arg Gln Ala Pro
30 35 40
GGC CAA GGG CTC GAA TGG ATT GGC TAT ATT GAT CCT TTC AAT GGT GGT 243
Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asp Pro Phe Asn Gly Gly
45 50 55
ACT AGC TAT AAT CAG AAG TTC AAG GGC AAG GTT ACC ATG ACC GTG GAC 291
Thr Ser Tyr Asn Gln Lys Phe Lys Gly Lys Val Thr Met Thr Val Asp
60 65 70
ACC TCT ACA AAC ACC GCC TAC ATG GAA CTG TCC AGC CTG CGC TCC GAG 339
Thr Ser Thr Asn Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu
75 80 85
GAC ACT GCA GTC TAC TAC TGC GCC AGA GGG GGT AAC CGC TTT GCT TAC 387
Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Gly Asn Arg Phe Ala Tyr
90 95 100 105

TGG GGA CAG GGT ACC CTT GTC ACC GTC AGT TCA GGTGAGTGGA TCC 433
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

SEQ. ID NO : 86

SEQUENCE LENGTH : 90

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GATAAGCTTG CCGCCACCAT GGACTGGACC TGGAGGGTCT TCTTCTTGCT 50
GGCTGTAGCT CCAGGTGCTC ACTCCCAGGT GCAGCTTGTG 90

SEQ. ID NO : 87

SEQUENCE LENGTH : 90

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

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GGCCTCAGTG AAGGTTTCCT GCAAGGCTTC TGGATACTCA 90

SEQ. ID NO : 88

SEQUENCE LENGTH : 90

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

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CCAGGCCCCC GGACAAAGGC TTGACTGGAT GGGATATATT	90

SEQ. ID NO : 89

SEQUENCE LENGTH : 90

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

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TCAGAAGTTC AAGGGCAGAG TCACCATTAC CGTAGACACA	90

SEQ. ID NO : 90

SEQUENCE LENGTH : 90

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

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CAGCCTGAGA TCTGAAGACA CGGCTGTGTA TTACTGTCCC	90

SEQ. ID NO : 91

SEQUENCE LENGTH : 94

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

ACGGCTGTGT ATTACTGTGC GAGAGGGGCT AACCGCTTTG CTTACTGGGG	50
CCAGGGAAACC CTGGTCACCG TCTCCTCAGG TGAGTGGATC CGAC	94

SEQ. ID NO : 92

SEQUENCE LENGTH : 15

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

CATAAGCTTG CCGCC	15
------------------	----

SEQ. ID NO : 93

SEQUENCE LENGTH : 15

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GTCCGATCCA CTCAC	15
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SEQ. ID NO : 94

SEQUENCE LENGTH : 433

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Double

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

ORIGINAL SOURCE

ORGANISM : Mouse and Human

IMADIATE SOURCE

CLONE : pUC-RV_H -sle 1220Ha

FEATURE : gene, excluding intron, coding for H chain V region version

"a" of reshaped human sleAUK1220 antibody to human IL-6R

amino acid -19--1:leader

amino acid 1 - 30 : FR1

amino acid 31 - 35 : CDR1

amino acid 36 - 49 : FR2

amino acid 50 - 66 : CDR2

amino acid 67 - 98 : FR3

amino acid 99 - 105:CDR3

amino acid 109 - 116:FR4

nucleotide 1 - 6 : Hind III site

nucleotide 427 - 433: Bam HI site

SEQUENCE

AACCTTGGCG CCACC ATC GAC TGG ACC TGG AGG GTC TTC TTG CTG GCT 51

Met Asp Trp Thr Trp Arg Val Phe Phe Leu Leu Ala

GTA GCT CCA CGT CCT CAC TCC CAG GTG CAG CTT GTG CAG TCT GGA GCT 99
Val Ala Pro Gly Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Ala
-5 -1 1 5
GAG GTG AAG AAG CCT GGG GCC TCA GTG AAG GTT TCC TGC AAG GCT TCT 147
Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser
10 15 20 25
GGA TAC TCA TTC ACT AGT TAT TAC ATA CAC TGG GTG CCC CAG CCC CCC 195
Gly Tyr Ser Phe Thr Ser Tyr Tyr Ile His Trp Val Arg Gln Ala Pro
30 35 40
GGA CAA AGG CTT GAG TGG ATG GGA TAT ATT GAC CCT TTC AAT GGT GGT 243
Gly Gln Arg Leu Glu Trp Met Gly Tyr Ile Asp Pro Phe Asn Gly Gly
45 50 55
ACT AGC TAT AAT CAG AAG TTC AAG GGC ACA GTC ACC ATT ACC GTC GAC 291
Thr Ser Tyr Asn Gln Lys Phe Lys Gly Arg Val Thr Ile Thr Val Asp
60 65 70
ACA TCC GCG AGC ACA GCC TAC ATG GAG CTG AGC AGT CTG AGA TCT GAA 339
Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu
75 80 85
GAC ACG GCT GTG TAT TAC TGT GCG AGA GGG GGT AAC CGC TTT GCT TAC 387
Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Gly Asn Arg Phe Ala Tyr
90 95 100 105
TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA GGTGAGTGGAA TCC 433
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110 115

SEQ. ID NO : 95

SEQUENCE LENGTH : 27

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

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27

SEQ. ID NO : 96

SEQUENCE LENGTH : 27

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

AAGTTCAAGG GCAAGGTAC CATTACC

27

SEQ. ID NO : 97

SEQUENCE LENGTH : 30

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

GGTGCTTCCG TGAAAGTCAG CTGTAAAGCT

30

SEQ. ID NO : 98

SEQUENCE LENGTH : 30

SEQUENCE TYPE : Nucleic acid

STRANDNESS : Single

TOPOLOGY : Linear

MOLECULE TYPE : Synthetic DNA

SEQUENCE

AGCTTTACAG CTGACTTTCA CGGAAGCACC

30

CLAIMS

1. A light chain (L chain) variable region (V region) of mouse monoclonal antibody to the human interleukin-6 receptor (IL-6R).
5 2. An L chain V region according to claim 1, having an amino acid sequence shown in any one of SEQ ID NOs: 24, 26, 28 and 30.
3. A heavy chain (H chain) V region of a mouse monoclonal antibody to the human IL-6R.
10 4. An H chain V region according to claim 3, having an amino acid sequence shown in SEQ ID NOs: 25, 27, 29 and 31.
5. A chimeric antibody to the human IL-6R, comprising:
 - 15 (1) an L chain comprising a human L chain constant region (C region) and an L chain V region of a mouse monoclonal antibody to the human IL-6R; and
 - (2) an H chain comprising a human H chain C region and an H chain V region of a mouse monoclonal antibody to the human IL-6R.
20
6. A chimeric antibody according to claim 5, wherein the mouse L chain V region has an amino acid sequence shown in any one of SEQ ID NOs: 24, 26, 28 and 30; and the mouse H chain V region has an amino acid sequence shown in any one of SEQ ID NOs: 25, 27, 29 and 31.
25
7. Complementarity determining regions (CDRs) of an L chain V region of a mouse monoclonal antibody to the human IL-6R.
30 8. CDR according to claim 7, having amino acid sequence shown in any one of SEQ ID NOs: 24, 26, 28 and 30 wherein the stretch of the amino acid sequence is defined in Table 9.
9. CDR of an H chain V region of a mouse monoclonal antibody to the human IL-6R.
35
10. CDR according to claim 9, having amino acid sequence shown in any one of SEQ ID NOs: 25, 27, 29, and

31 wherein the stretch of the amino acid sequence is defined in Table 9.

11. A reshaped human L chain V region of an antibody to the human IL-6R, comprising:

5 (1) framework regions (FRs) of a human L chain V region, and

(2) CDRs of an L chain V region of a mouse monoclonal antibody to the human IL-6R.

10 12. A reshaped human L chain V region according to claim 11, wherein the CDRs have amino acid sequences shown in any one of SEQ ID Nos.: 24, 26, 28 and 30 wherein the stretches of the amino acid sequences are defined in Table 9.

15 13. A reshaped human L chain V region according to claim 11, wherein the FRs are derived from the human antibody REI.

14. A reshaped human L chain V region according to claim 11, having an amino acid sequence shown as RV_La or RV_Lb in Table 2.

20 15. A reshaped human L chain V region according to claim 11, having an amino acid sequence shown as RV_L in Table 5.

16. A reshaped human H chain V region of an antibody to the human IL-6R, comprising:

25 (1) FRs of a human H chain V region, and

(2) CDRs of an H chain V region of a mouse monoclonal antibody to the human IL-6R.

30 17. A reshaped human H chain V region according to claim 16, wherein the CDRs have amino acid sequences shown in SEQ ID NOS: 25, 27, 29 and 31 wherein the stretches of the amino acid sequences are defined in Table 9.

35 18. A reshaped human H chain V region according to claim 16, wherein the FRs are derived from the human antibody NEW or HAX.

19. A reshaped human H chain V region according to

claim 16, having an amino acid sequence shown in Table 3 as RV_Ha, RV_Hb, RV_Hc, RV_Hd, RV_He or RV_Hf.

20. A reshaped human H chain V region according to claim 17, having an amino acid sequence shown as RV_Ha, 5 RV_Hb, RV_Hc or RV_Hd in Table 7.

21. An L chain of a reshaped human antibody to human IL-6R comprising:

(1) a human L chain C region; and

(2) an L chain V region comprising human

10 L chain FRs and L chain CDRs of mouse monoclonal antibody to human IL-6R.

22. A reshaped human antibody L chain according to claim 21, wherein the human L chain C region is a human γ -1C region, the human L chain FRs are derived from 15 REI, and the L chain CDRs have amino acid sequences shown in SEQ ID Nos. 24, 26, 28 and 30 wherein the stretches of the amino acid sequences are defined in Table 9.

23. A reshaped human antibody L chain according to claim 21, wherein the L chain V region has an amino acid 20 sequence shown as RV_La or RV_Lb in Table 2.

24. A reshaped human antibody L chain according to claim 21, wherein the L chain V region has an amino acid sequence shown as RV_L in Table 5.

25. An H chain of a reshaped human antibody to 25 human IL-6R comprising:

(1) a human H chain C region, and

(2) an H chain V region comprising human

H chain FRs, and H chain CDRs of mouse monoclonal antibody to human IL-6.

30 26. A reshaped human antibody H chain according to claim 25, wherein the human H chain C region is human κ C region, the human H chain FRs are derived from NEW or HAX, the H chain CDRs have amino acid sequences shown in SEQ ID NOS: 25, 27, 29 or 31 wherein the stretches of the 35 amino acid sequences are defined in Table 9.

27. A reshaped human antibody H chain according to

claim 25, wherein the H chain V region has an amino acid sequence shown as RV_Ha, RV_Hb, RV_Hc or RV_Hd in Table 3.

28. A reshaped human antibody H chain according
claim 25, wherein the H chain V region has an amino acid
5 sequence shown as RV_Ha, RV_Hb, RV_Hc or RV_Hd in Table 6, or
RV_Ha, RV_Hb, RV_Hc or RV_Hc in Table 7.

29. A reshaped antibody to the human IL-6R,
comprising:

- (A) an L chain comprising,
 - 10 (1) a human L chain C region, and
 - (2) an L chain V region comprising human L chain FRs, and L chain CDRs of a mouse monoclonal antibody to the human IL-6R; and
- (B) an H chain comprising,
 - 15 (1) a human H chain C region, and
 - (2) an H chain V region comprising human H chain FRs, and H chain CDRs of a mouse monoclonal antibody to the human IL-6R.

30. A reshaped human antibody according to
claim 29, wherein the L chain CDRs have amino acid
sequences shown in any one of SEQ ID NOS: 24, 26, 28 and
30 wherein the stretches of the amino acid sequences are
defined in Table 9; the H chain CDRs have amino acid
sequence shown in any one of SEQ ID NOS: 25, 27, 29 and
25 31 wherein the stretches of the amino acid sequences are
defined in Table 9; the human L chain C region and human
L chain FRs are derived from the REI; and the human H
chain C region and human FRs are derived from the NEW or
HAX.

30 31. A reshaped human antibody according to
claim 29, wherein the L chain V region has an amino acid
sequence shown as RV_La or RV_Lb in Table 2.

32. A reshaped human antibody according to
claim 29, wherein the L chain V region has an amino acid
35 sequence shown as RV_L in Table 5.

33. A reshaped human antibody according to

claim 29, wherein the H chain V region has an amino acid sequence shown in Table 3 as RV_Ha, RV_Hb, RV_Hc, RV_Hd, RV_He or RV_Hf.

34. A reshaped human antibody according to
5 claim 29, wherein the H chain V region has an amino acid sequence shown as RV_Ha, RV_Hb, RV_Hc or RV_Hd in Table 6, or RV_Ha, RV_Hb, RV_Hd in Table 7.

35. A DNA coding for an L chain V region of a mouse monoclonal antibody to the human IL-6R.

10 36. A DNA according to claim 35, wherein the L chain V region has an amino acid sequence shown in any one of SEQ ID NOs: 24, 26, 28 and 30.

37. A DNA coding for an H chain V region of a mouse monoclonal antibody to the human IL-6R.

15 38. A DNA according to claim 37, wherein the H chain V region has an amino acid sequence shown in any one of SEQ ID NOs: 25, 27, 29 and 31.

39. A DNA coding for CDR of an L chain V region of a mouse monoclonal antibody to the human IL-6R.

20 40. A DNA coding for CDR according to claim 39, wherein the CDR has an amino acid sequence in any one of SEQ ID NOs: 24, 26, 28 and 30 wherein the stretch of the amino acid sequence is defined in Table 9.

41. A DNA coding for CDR of an H chain V region of
25 a mouse monoclonal antibody to the human IL-6R.

42. A DNA coding for CDR according to claim 41, wherein the CDR has an amino acid sequence shown in any one of SEQ ID NOs: 25, 27, 29 and 31 wherein the stretch of the amino acid sequence is defined in Table 9.

30 43. A DNA coding for a reshaped human L chain V region of an antibody to the human IL-6R, wherein the reshaped human L chain V region comprises:

(1) FRs of a human L chain V region, and
35 (2) CDRs of a mouse L chain V region of a monoclonal antibody to the human IL-6R.

44. A DNA coding for a reshaped human L chain

V region according to claim 43, wherein the CDRs have amino acid sequences shown in any one of SEQ ID NOS: 24, 26, 28 and 30 wherein the stretches of the amino acid sequences are defined in Table 9.

5 45. A DNA coding for a reshaped human L chain V region according to claim 43, wherein the FRs are derived from the REI.

10 46. A DNA according to claim 43, wherein the L chain V region has an amino acid sequence shown as RV_La or RV_Lb in Table 2.

47. A DNA according to claim 43, wherein the L chain V region has an amino acid region shown as RV_L in Table 5.

15 48. A DNA according to claim 43, having a nucleotide sequence shown in SEQ ID No: 57.

49. A DNA coding for a reshaped human H chain V region of an antibody to the human IL-6R, wherein the reshaped Human V region comprises:

20 (1) FRs of a human H chain V region, and
(2) CDRs of an H chain V region of a mouse monoclonal antibody to the human IL-6R.

25 50. A DNA coding for a reshaped human H chain V region according to claim 49, wherein the CDRs have amino acid sequences shown in SEQ ID NOS: 25, 27, 29 and 31 wherein the stretches of the amino acid sequences are defined in Table 9.

51. A DNA coding for a reshaped human H chain V region according to claim 49, wherein the FRs are derived from the NEW or HAX.

30 52. A DNA coding for a reshaped human H chain V region according to claim 49, wherein the H chain V region has an amino acid sequence shown as RV_Ha, RV_Hb, RV_Hc, RV_Hd, RV_He or RV_Hf in Table 3.

35 53. A DNA according to claim 49, wherein the H chain V region has an amino acid sequence shown as RV_La, RV_Hb, RV_Hc or RV_Hd in Table 6, or RV_Ha, RV_Hb, RV_Hc or

RV_Hd in Table 7.

54. A DNA according to claim 49, having a nucleotide sequence shown in SEQ ID NO: 56.

5 55. A DNA coding for a reshaped human L chain of an antibody to the human IL-6R, wherein the reshaped human L chain comprises:

10 (1) a human L chain C region; and
(2) an L chain V region comprising a human FRs, and CDRs of a mouse monoclonal antibody to the human IL-6R.

56. A DNA according to claim 55, wherein the L chain V region has the nucleotide sequence shown in SEQ ID NO: 57.

15 57. A DNA coding for a reshaped human H chain of an antibody to the human IL-6R, wherein the reshaped human H chain comprises:

20 (1) a human H chain C region, and
(2) a H chain V region comprising human FRs, and CDRs of a mouse monoclonal antibody to the human IL-6R.

58. A DNA according to claim 57, wherein the H chain V region has the nucleotide sequence shown in SEQ ID NO: 56.

25 59. A vector comprising a DNA according to any one of claims 35, 37, 39, 41, 43, 49, 55 and 57.

60. A host cell transformed or transfected with a vector comprising a DNA according to any one of claims 35, 37, 39, 41, 43, 49, 55 and 57.

30 61. A DNA coding for a chimeric L chain of an antibody to the human IL-6R, wherein the chimeric L chain comprises:

(1) a human L chain C region; and
(2) an L chain V region of a mouse monoclonal antibody to the human IL-6R.

35 62. A DNA coding for a chimeric H chain of an antibody to the human IL-6R wherein the chimeric H chain

comprises:

- (1) a human H chain C region; and
- (2) an H chain V region of a mouse monoclonal antibody to the human IL-6R.

5 63. A process for production of a chimeric antibody to the human IL-6R, comprising the steps of:

culturing host cells cotransfected with an expression vector comprising a DNA according to claim 61 and with an expression vector comprising a DNA according

10 to claim 62; and

recovering a desired antibody.

64. A process for production of a reshaped human antibody to the human IL-6R, comprising the steps of:

culturing host cells cotransfected with an expression vector comprising a DNA according to claim 55 and with an expression vector comprising a DNA according to claim 57; and recovering desired antibody.

65. A DNA according to claim 49, having a nucleotide sequence shown in SEQ ID NO: 85, 86 or 94.

20 66. A DNA according to claim 44, having a nucleotide sequence shown in SEQ IN NO: 71.

ABSTRACT

A reshaped human antibody to the human IL-6R, comprising:

5 (A) an L chain comprising,

(1) a human L chain C region, and

(2) an L chain V region comprising human L chain framework regions (FRs), and mouse L chain complementary determination regions (CDRs) of a monoclonal antibody to the IL-6 receptor (IL-6R); and

10 (B) an H chain comprising,

(1) a human H chain C region, and

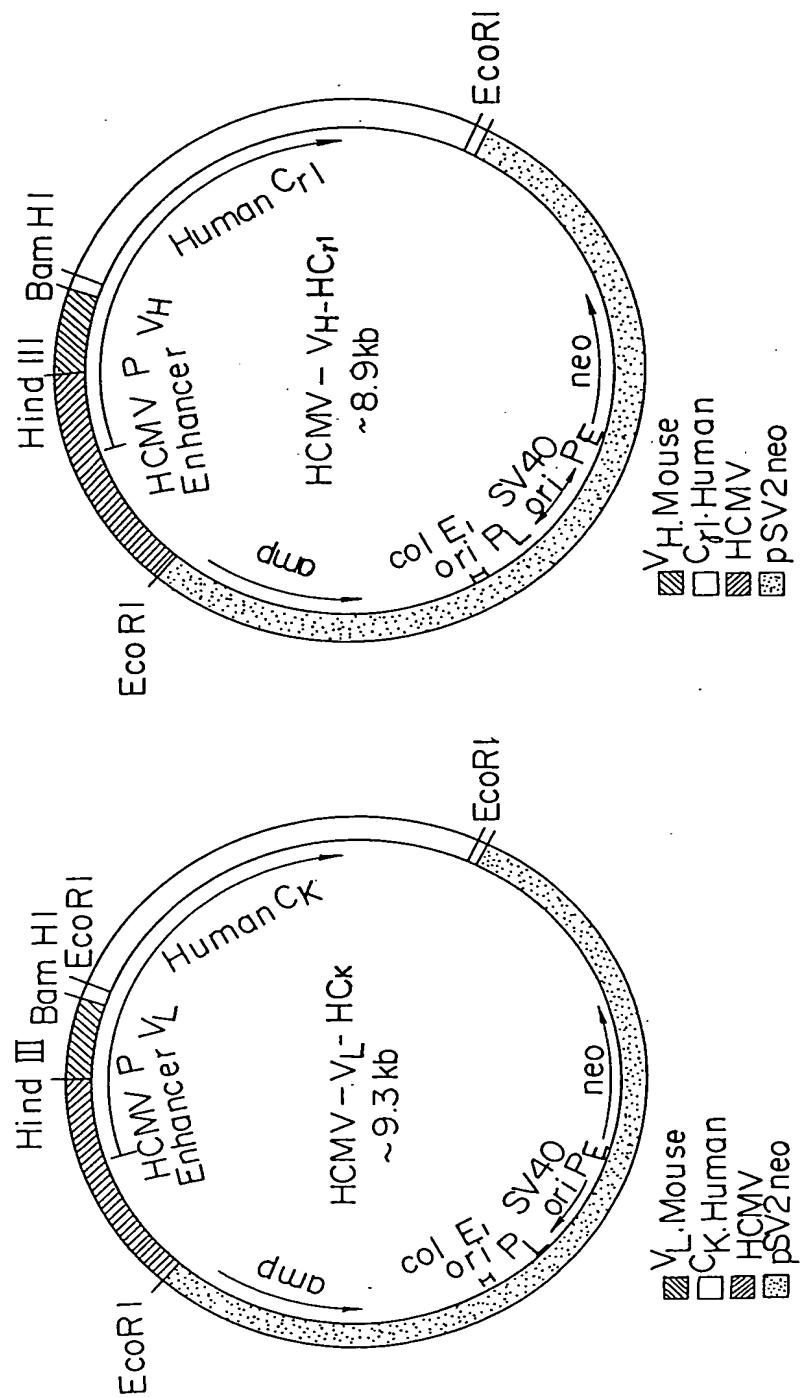
(2) an H chain V region comprising human H chain FRs, and mouse H chain CDRs of a monoclonal antibody to the IL-6R.

15 Since major portion of the reshaped human antibody is derived from a human antibody and the mouse CDRs which are less immunogenic, the present reshaped human antibody is less immunogenic to human, and therefor is promised for therapeutic uses.



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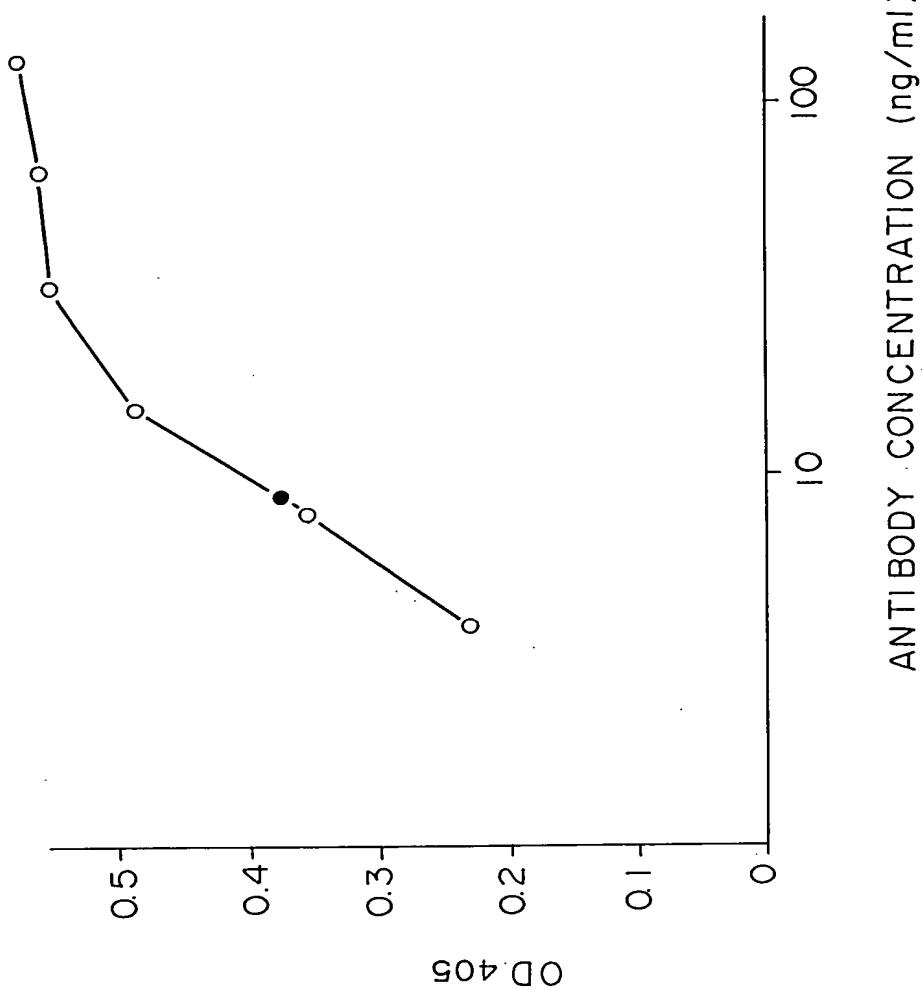
Fig. 1





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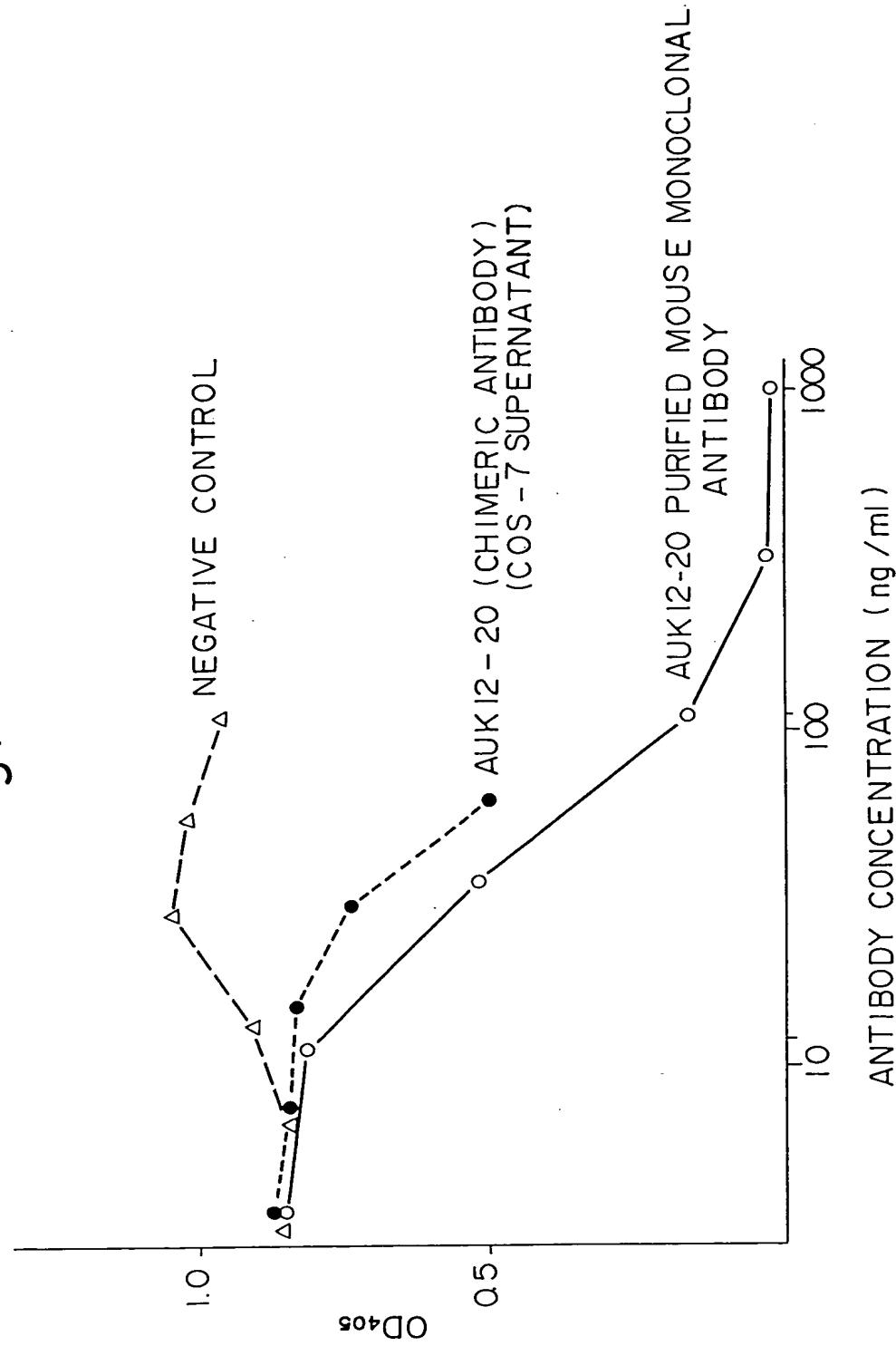
Fig. 2





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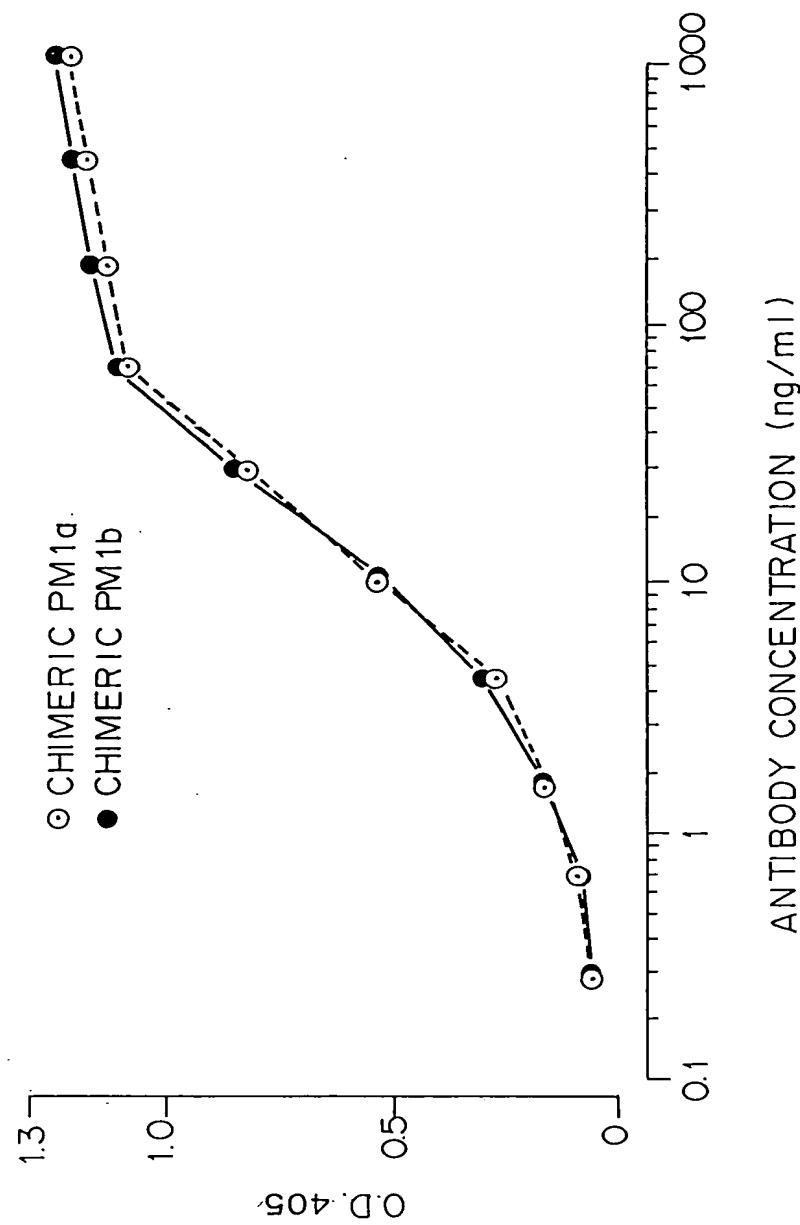
Fig. 3





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Fig. 4



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Fig. 5

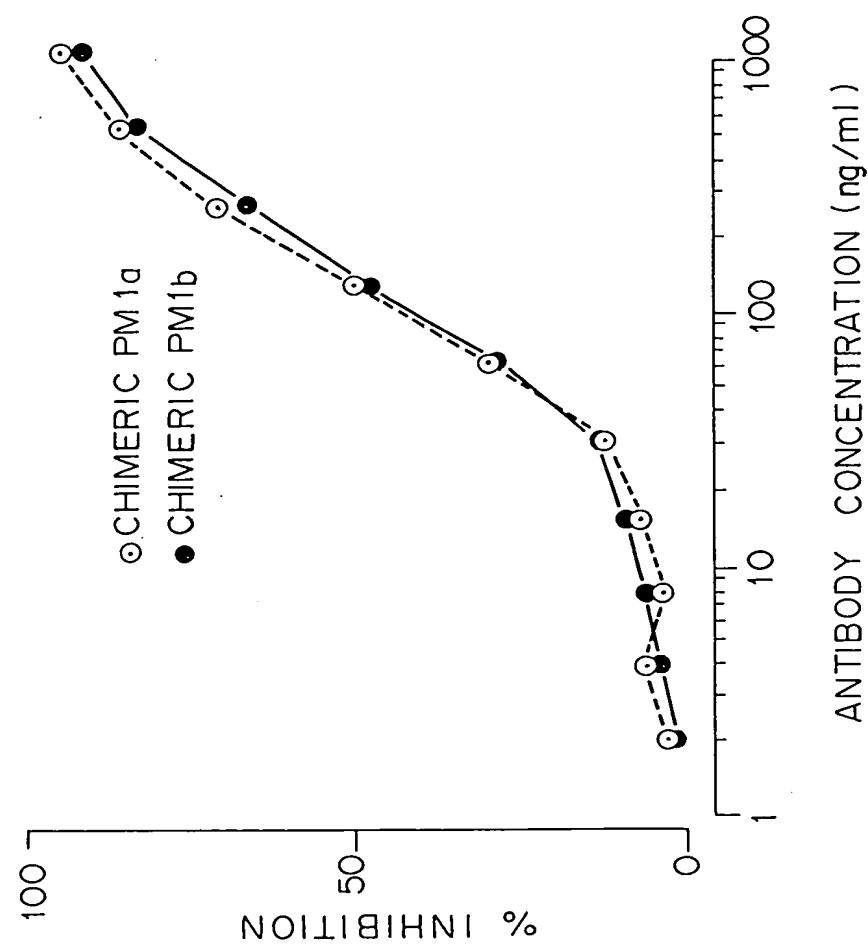




Fig. 6

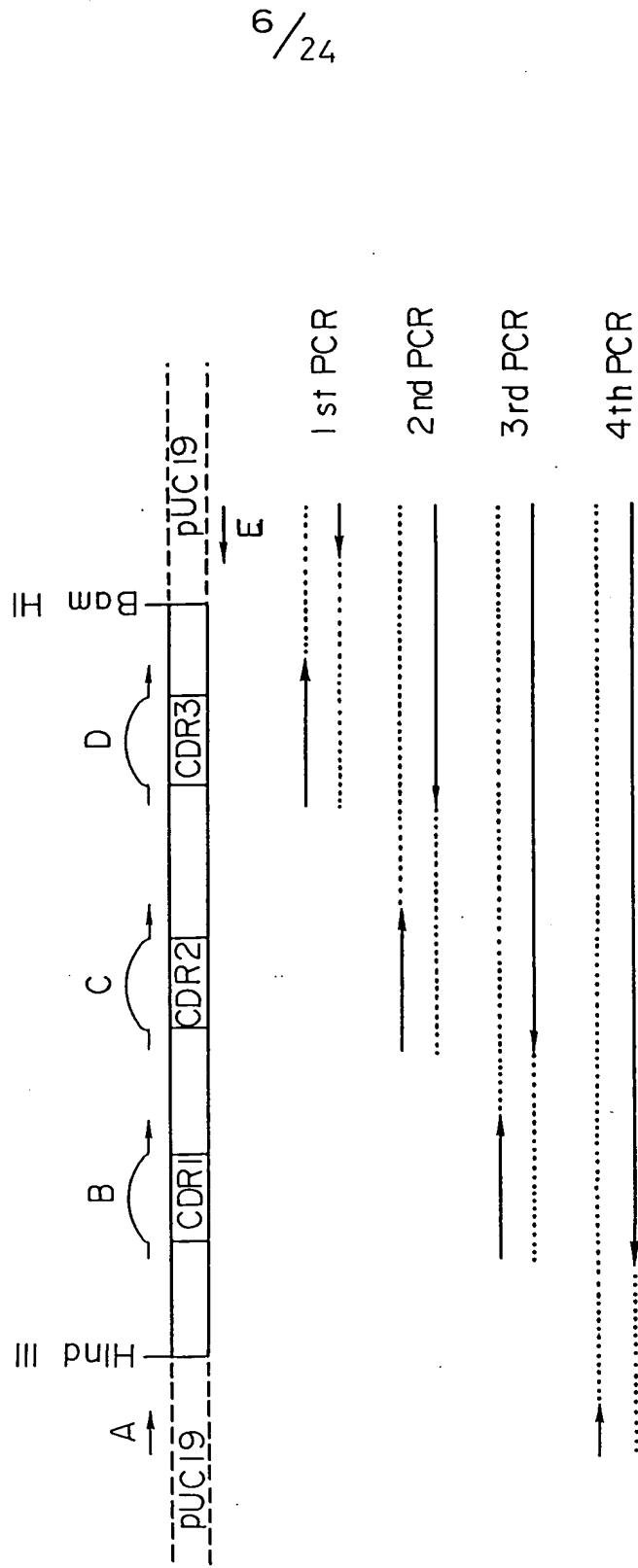


Fig. 7

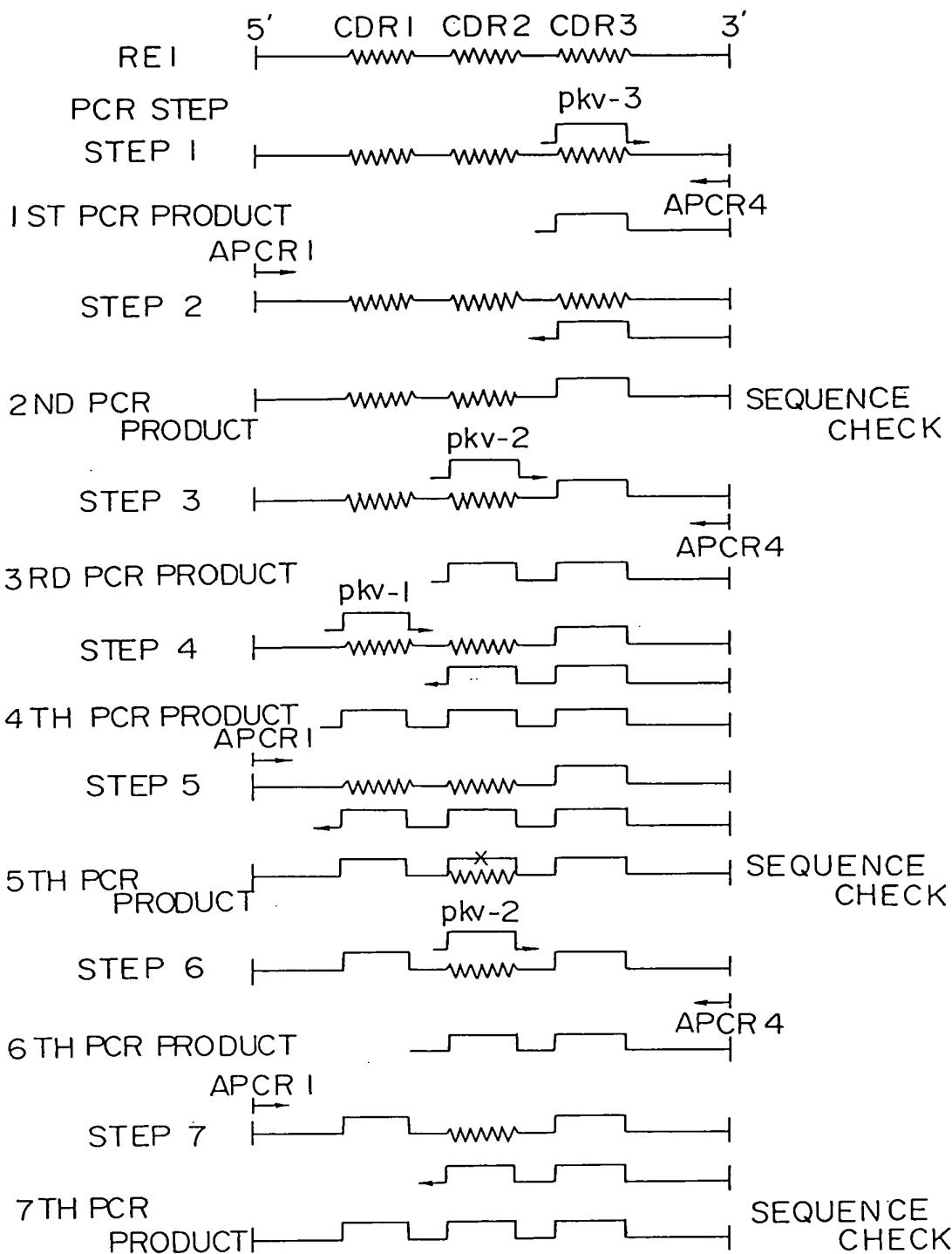


Fig. 8

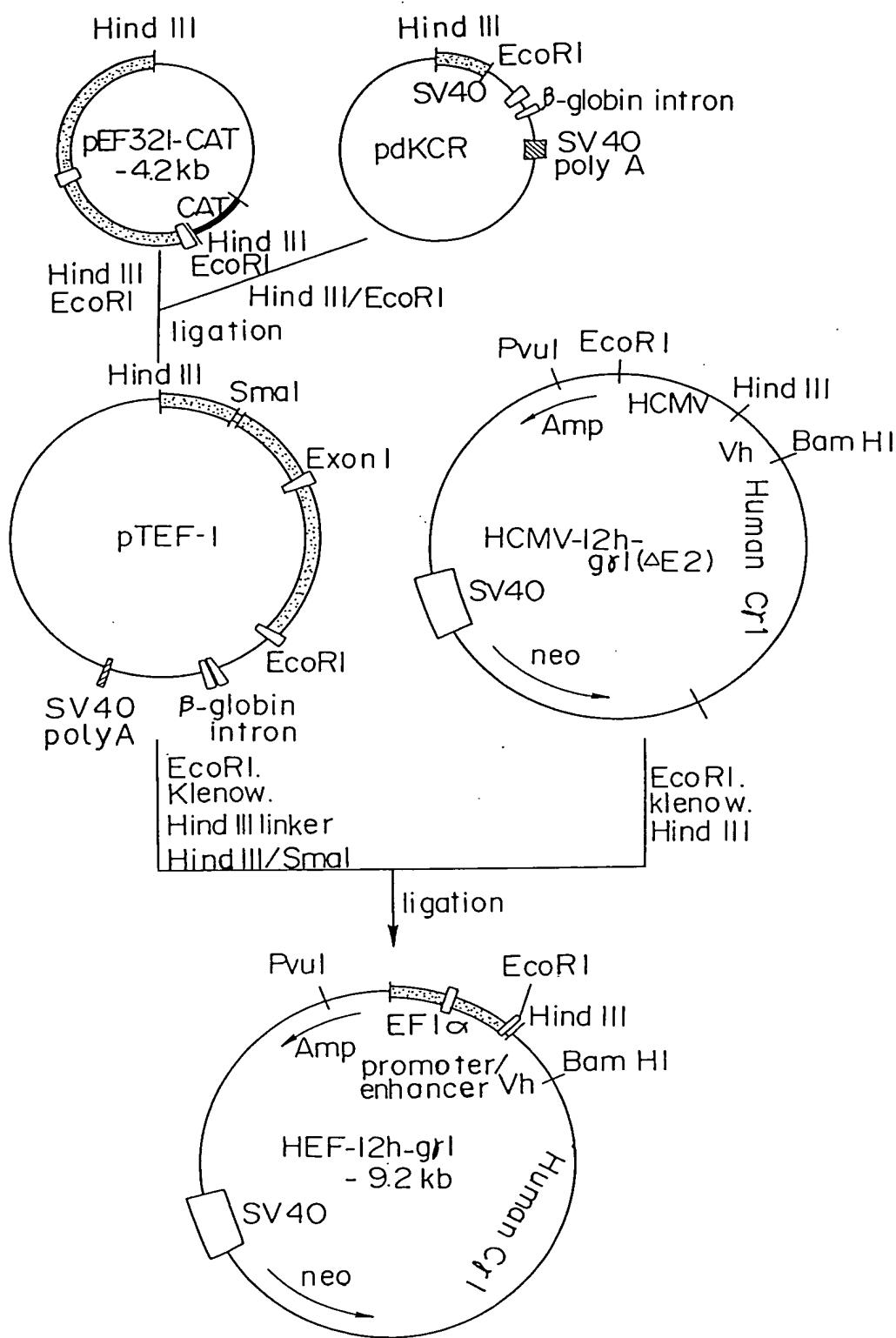


Fig. 9

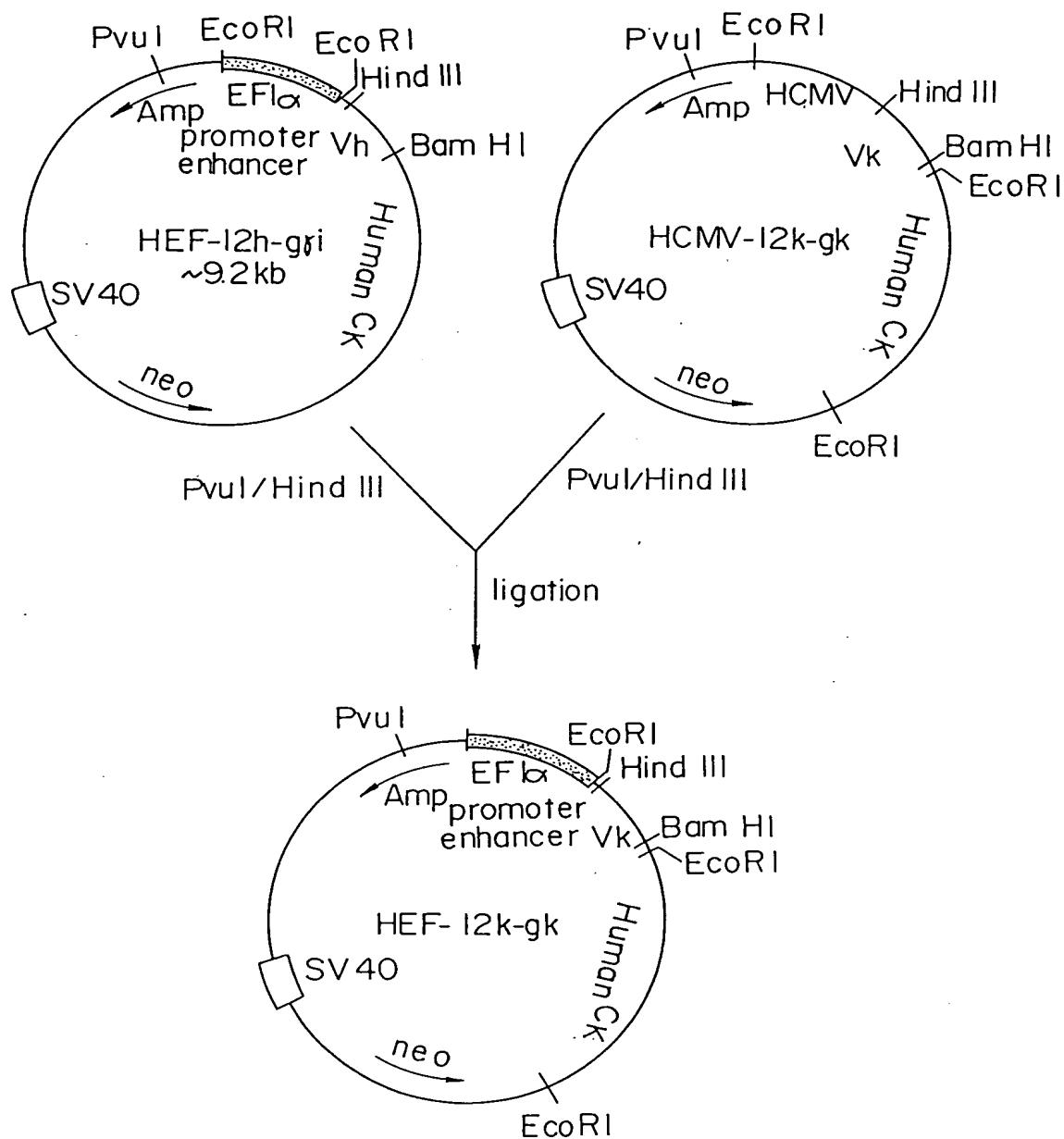


Fig. 10

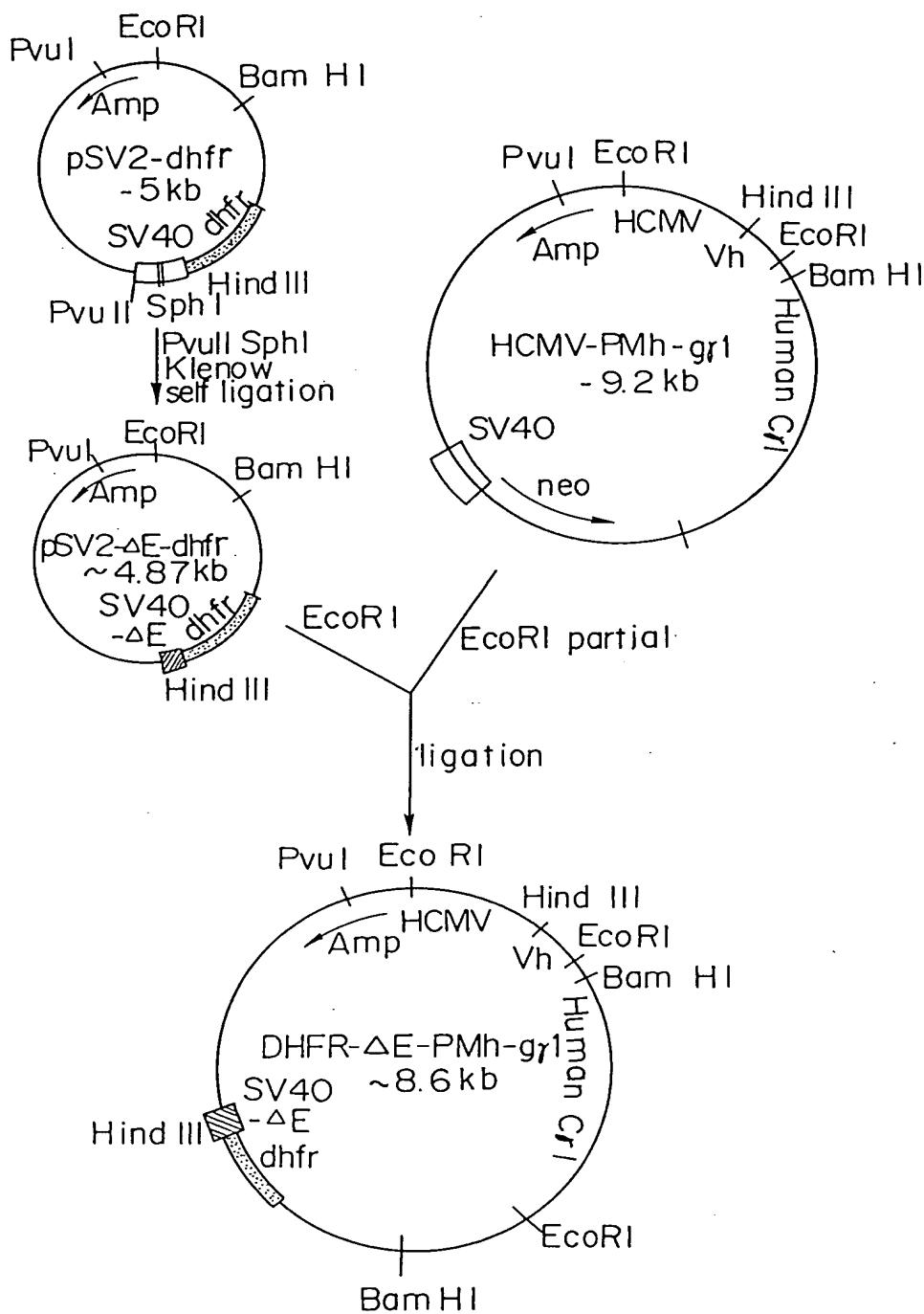
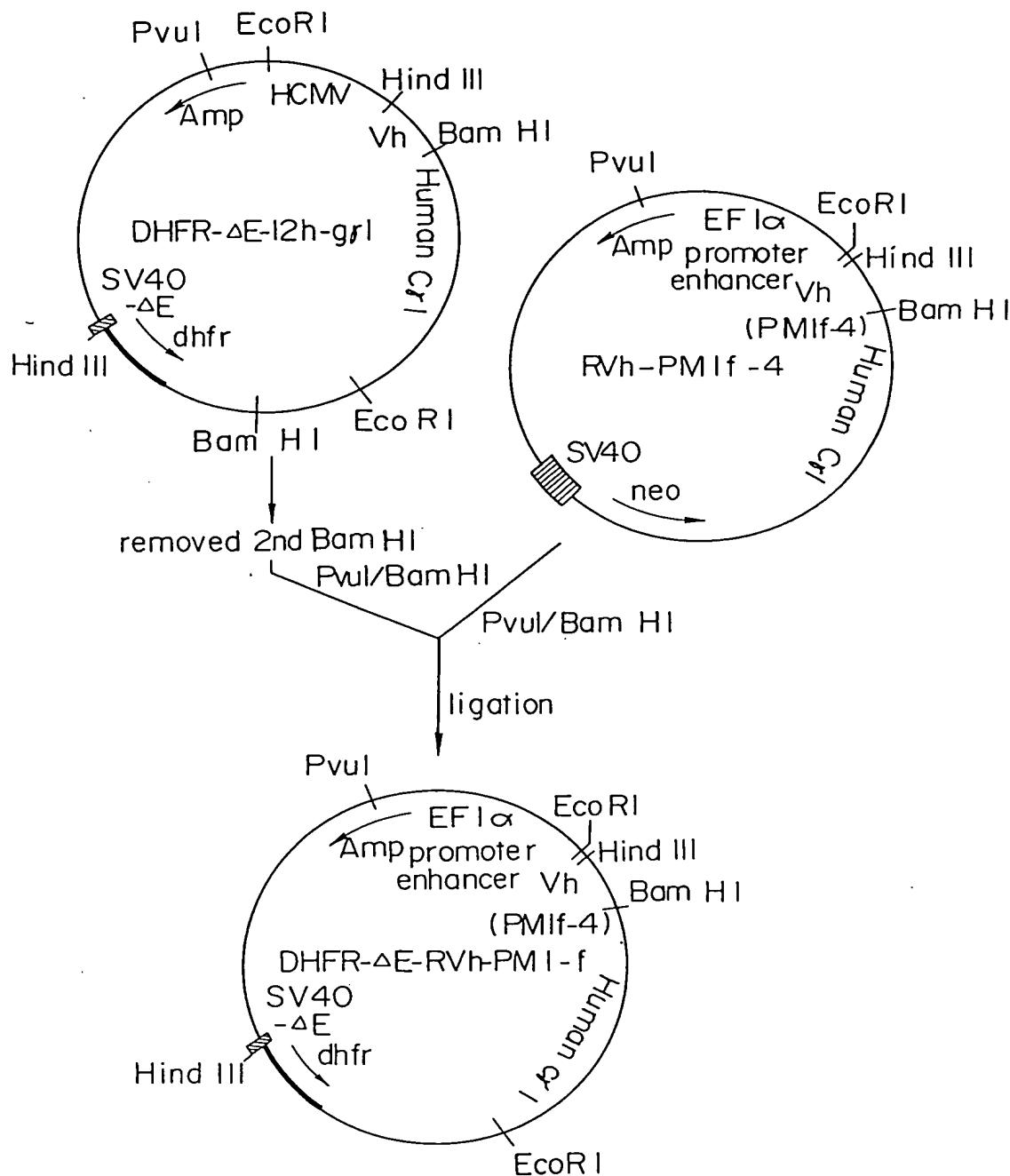


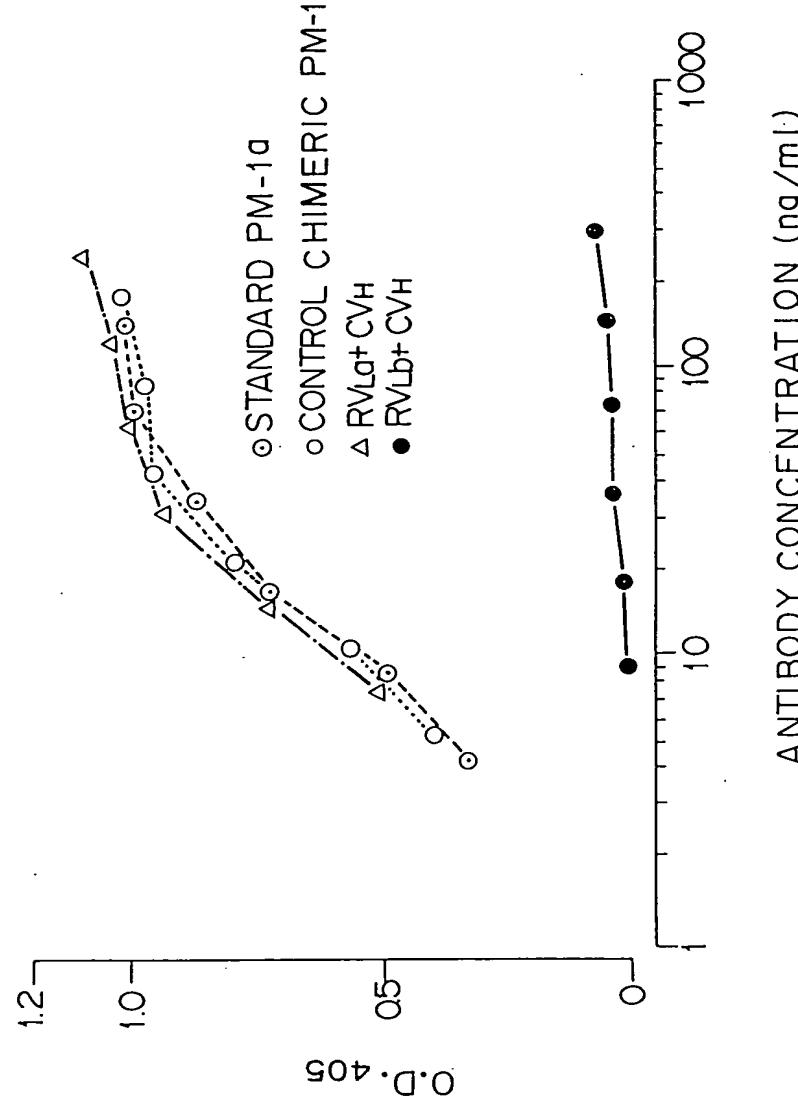
Fig. 11





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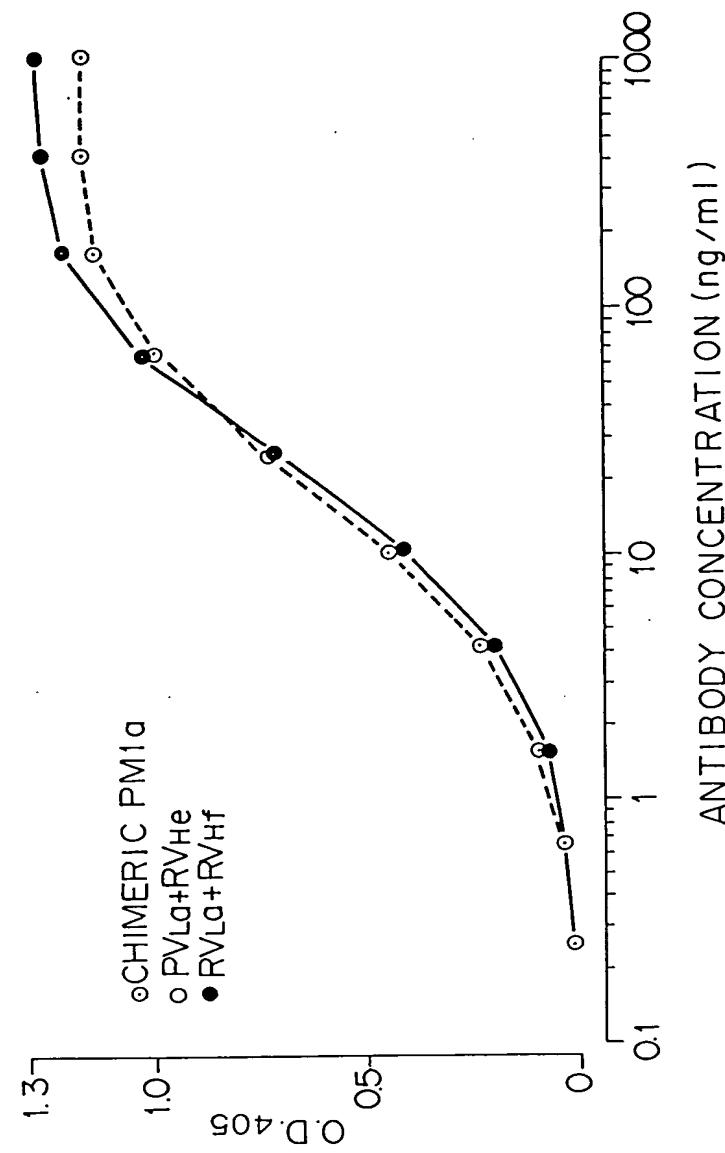
Fig. 12





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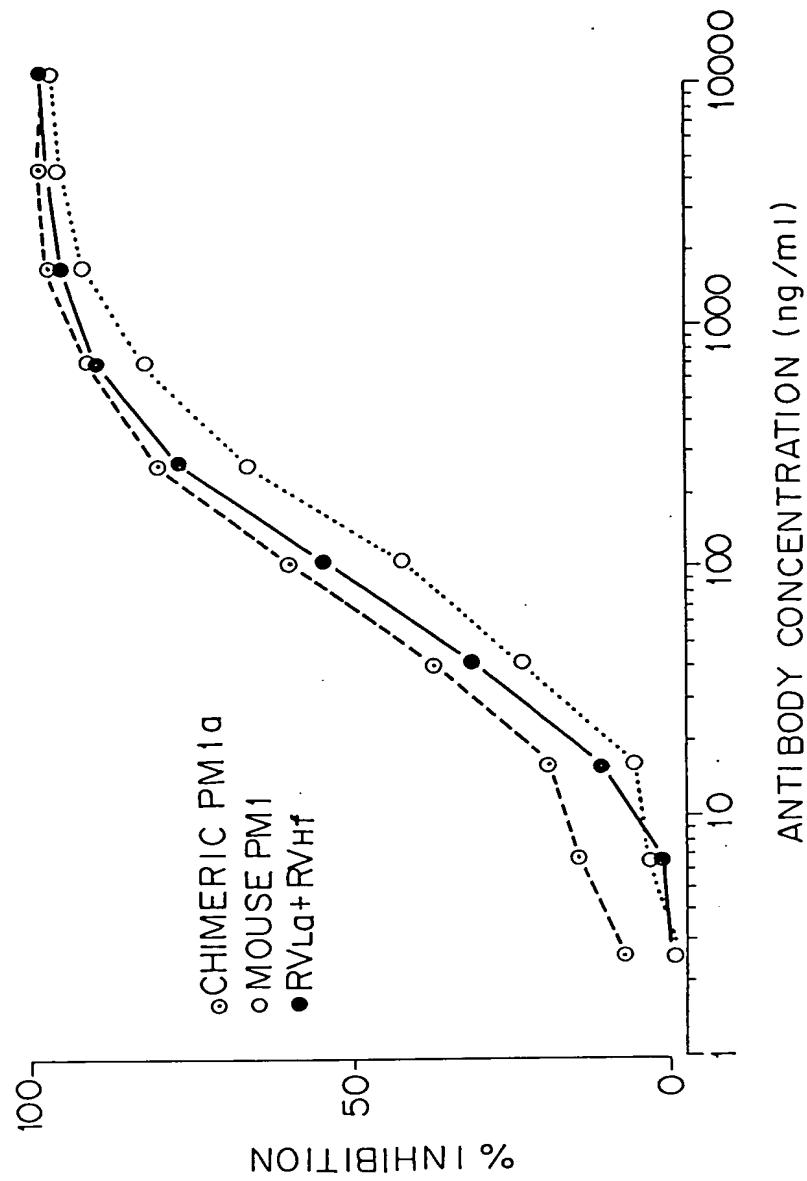
Fig. 13





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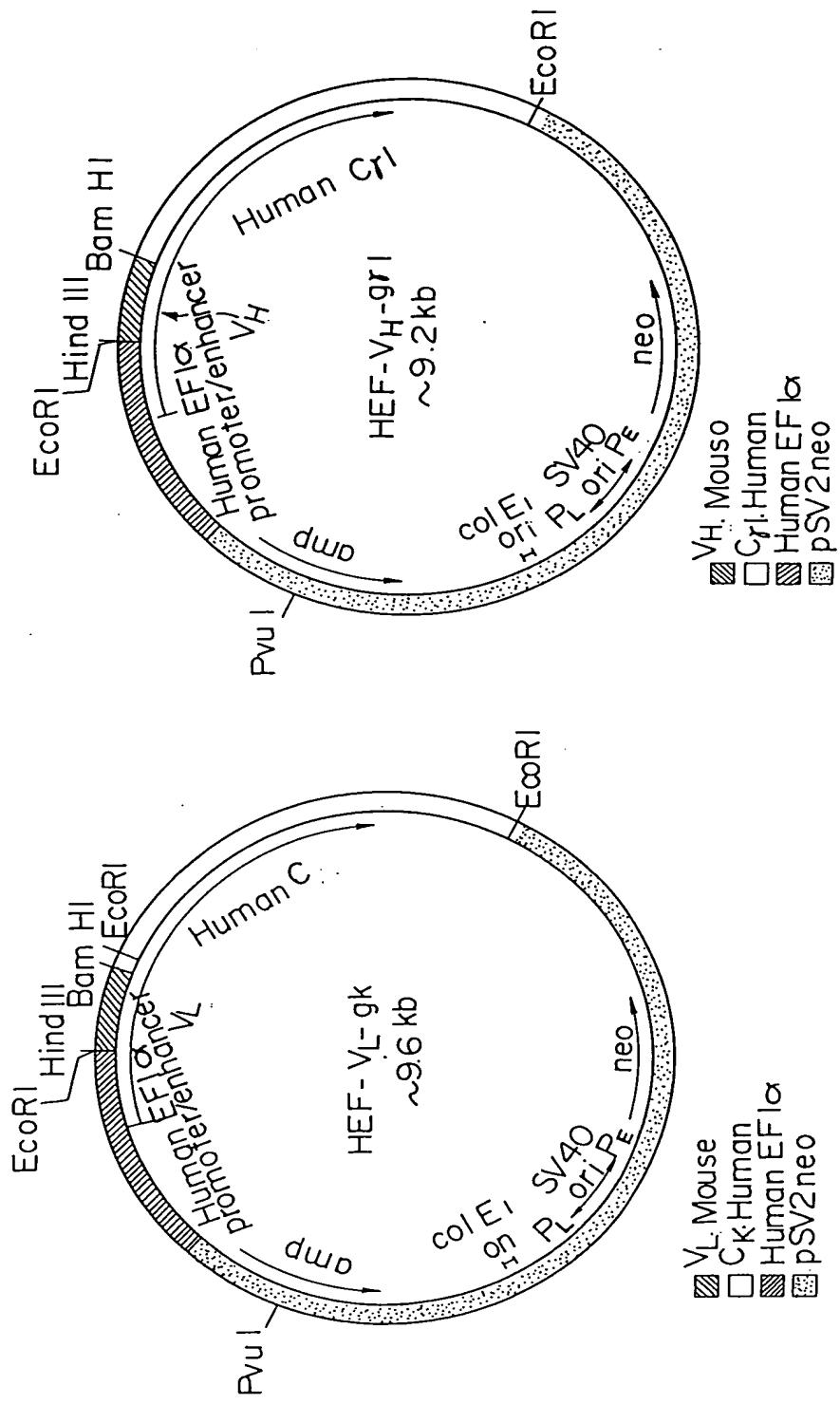
Fig. 14





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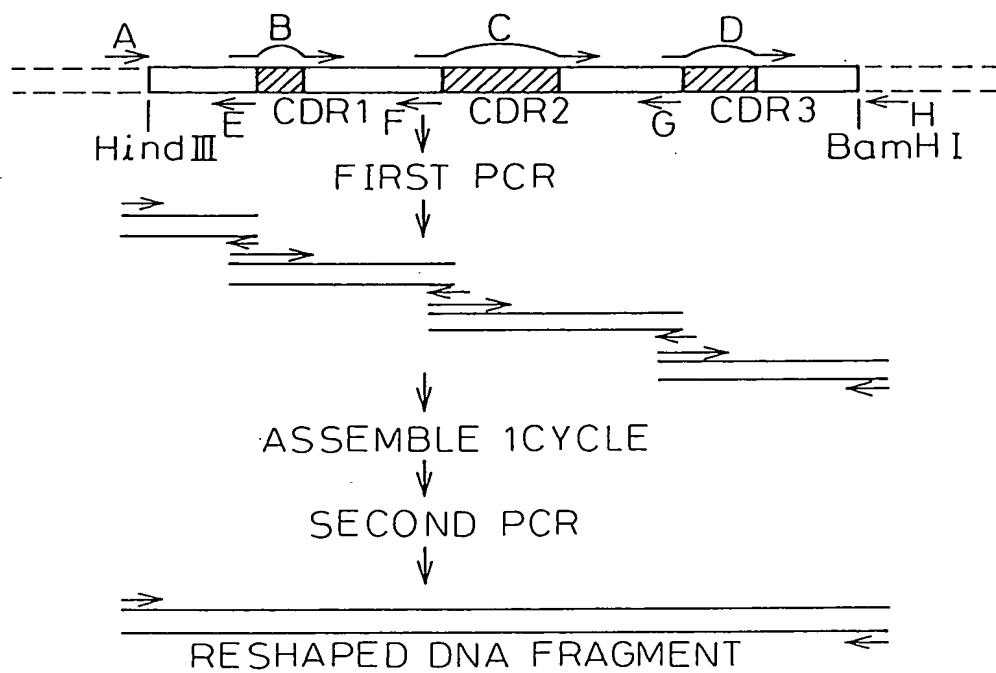
Fig. 15





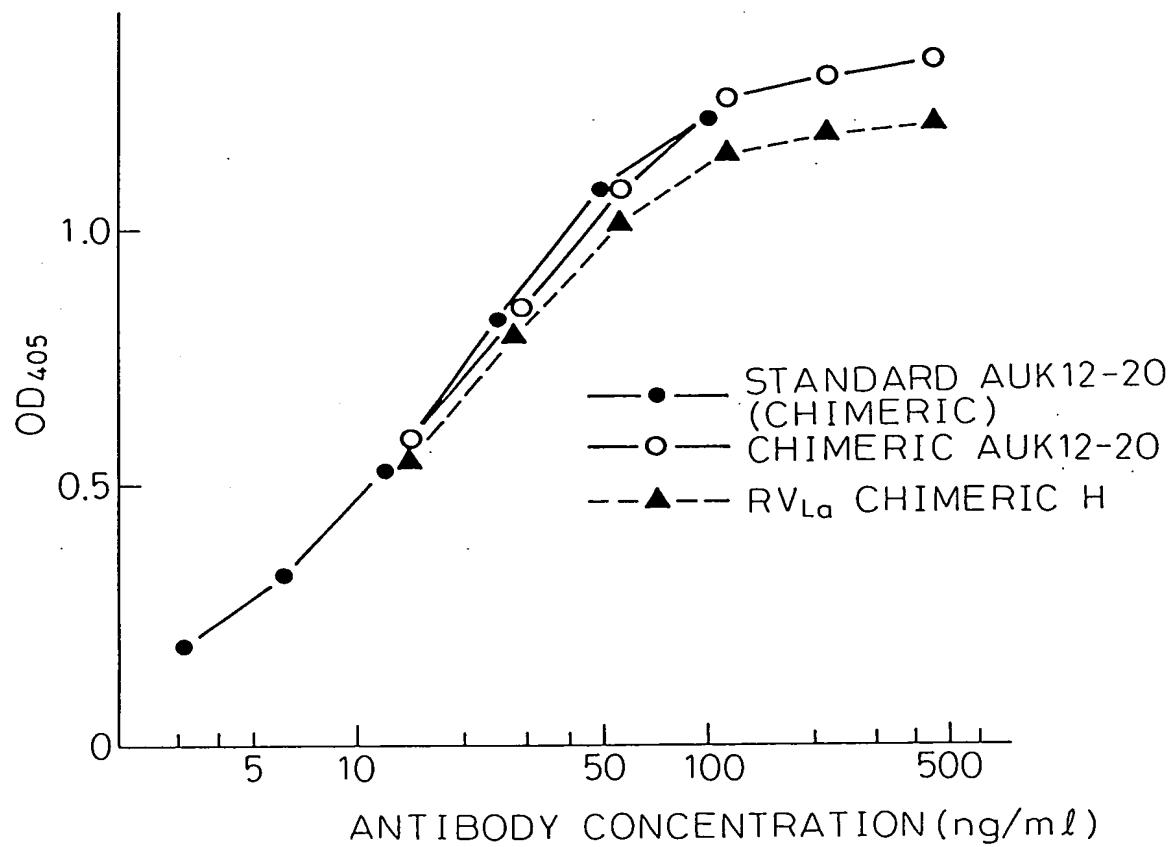
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Fig.16



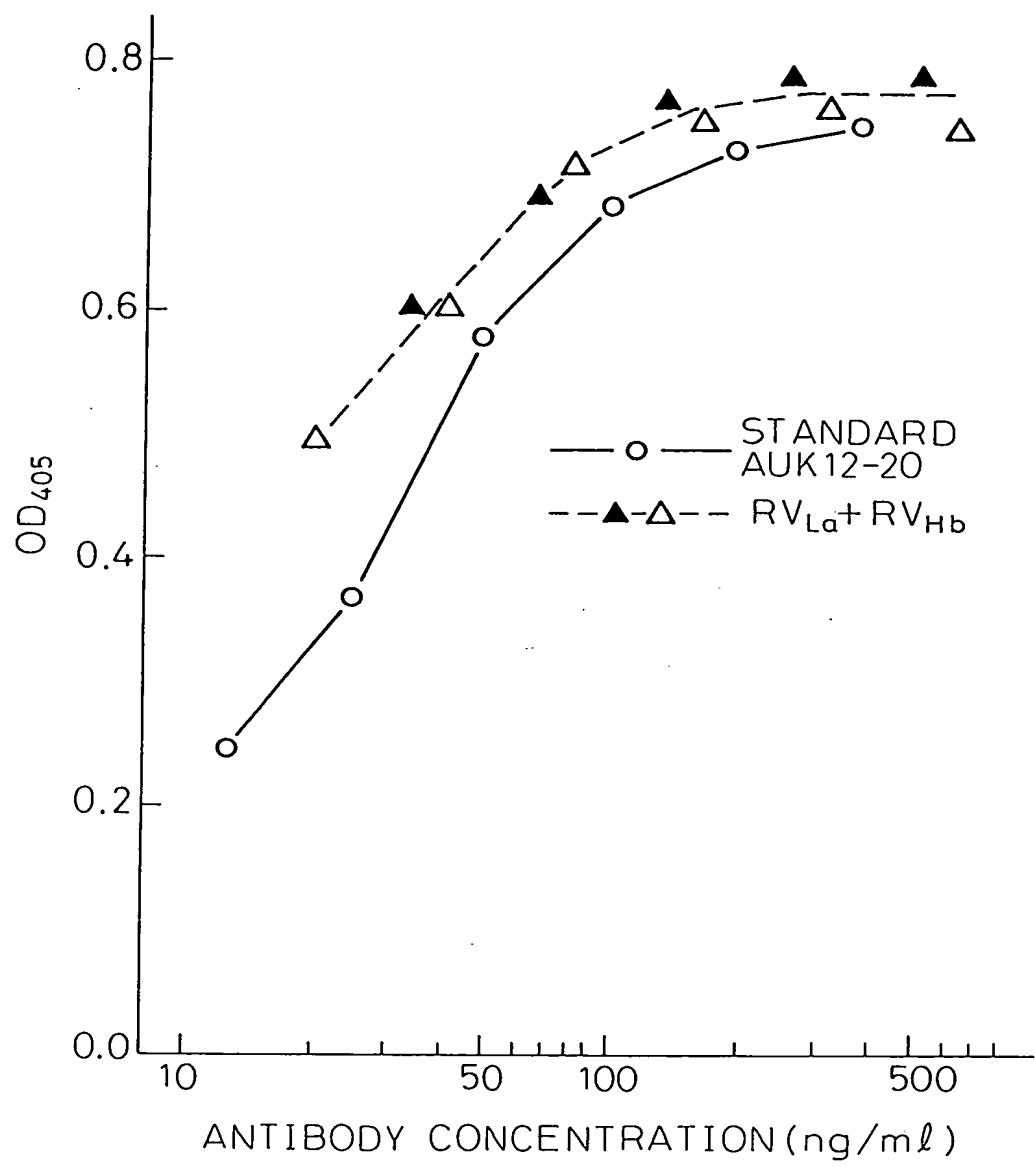
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Fig.17



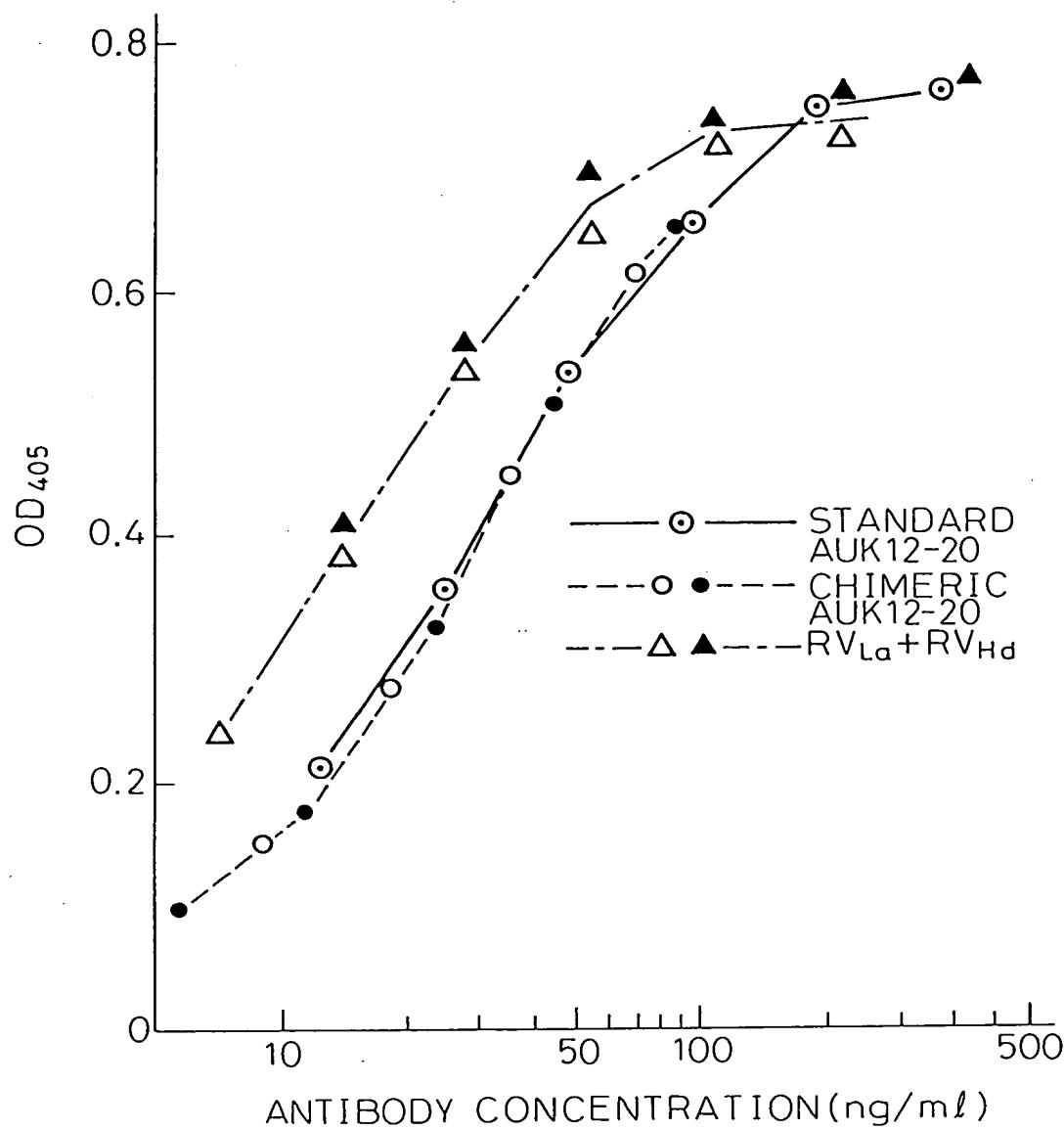
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Fig.18



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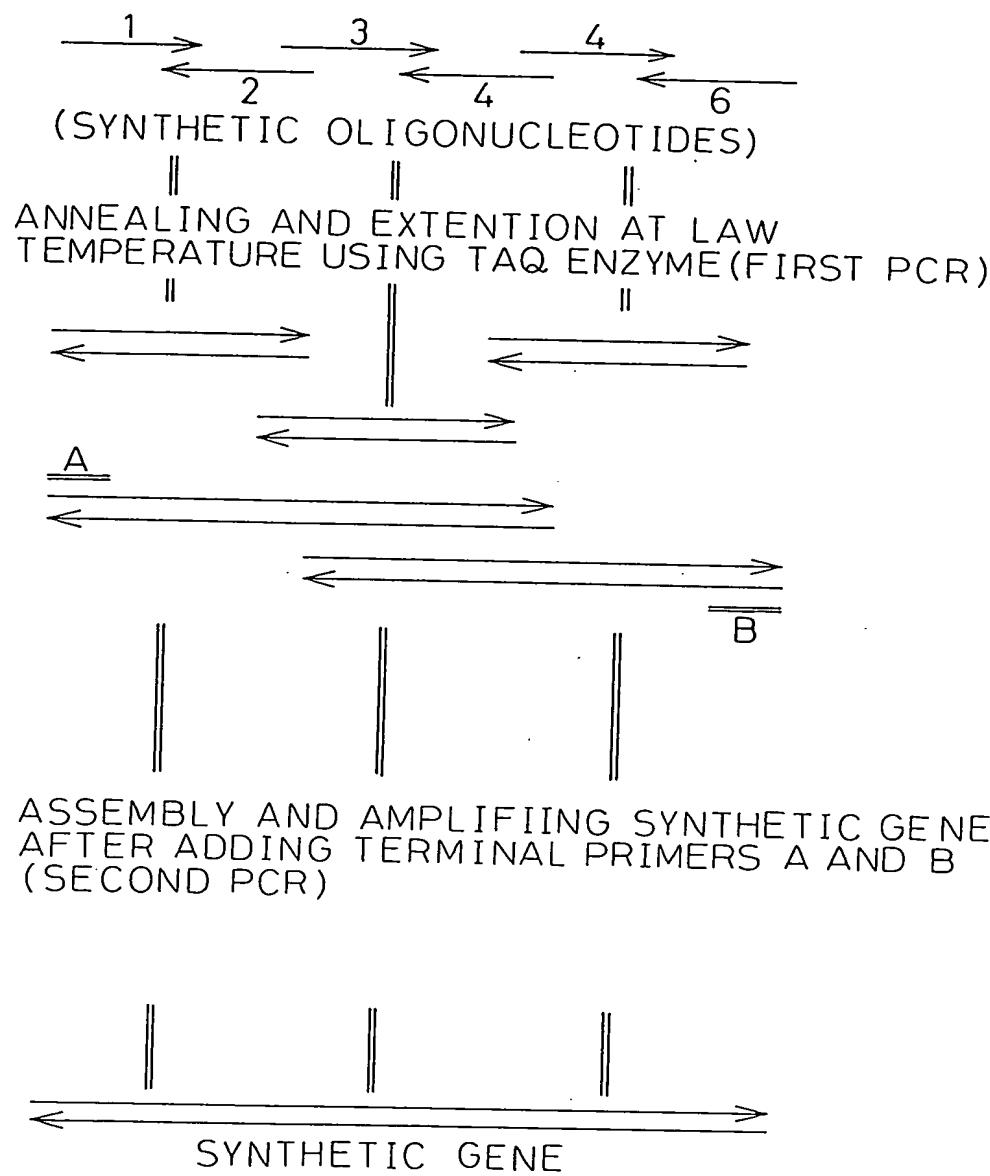
Fig.19





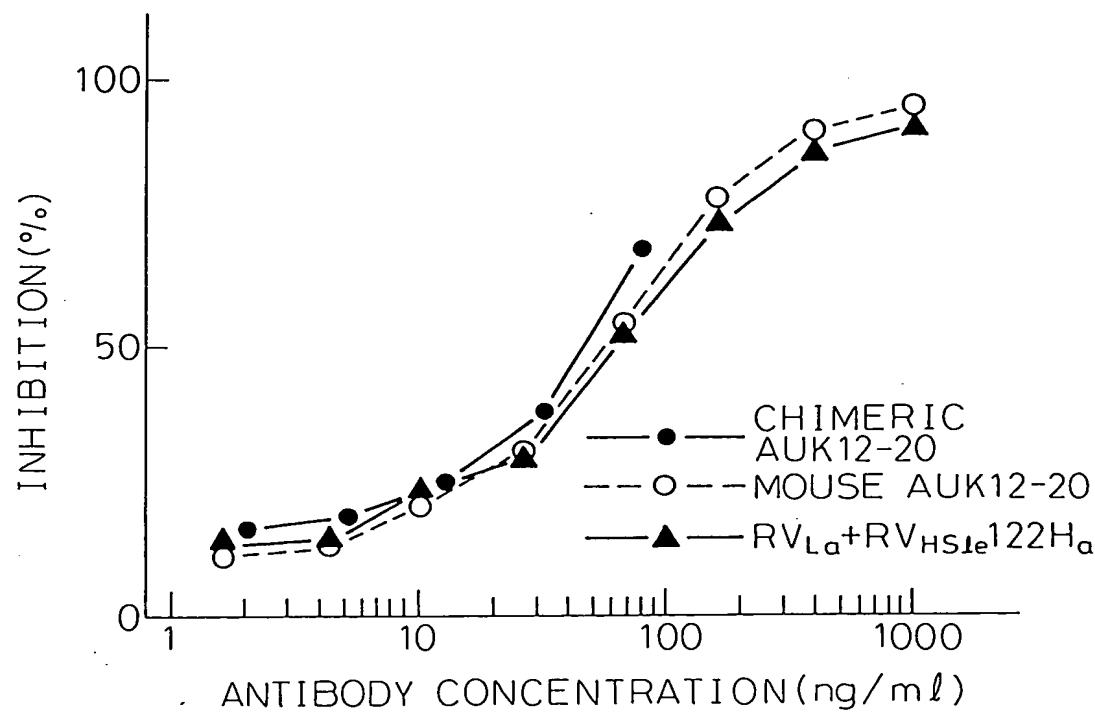
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Fig.20



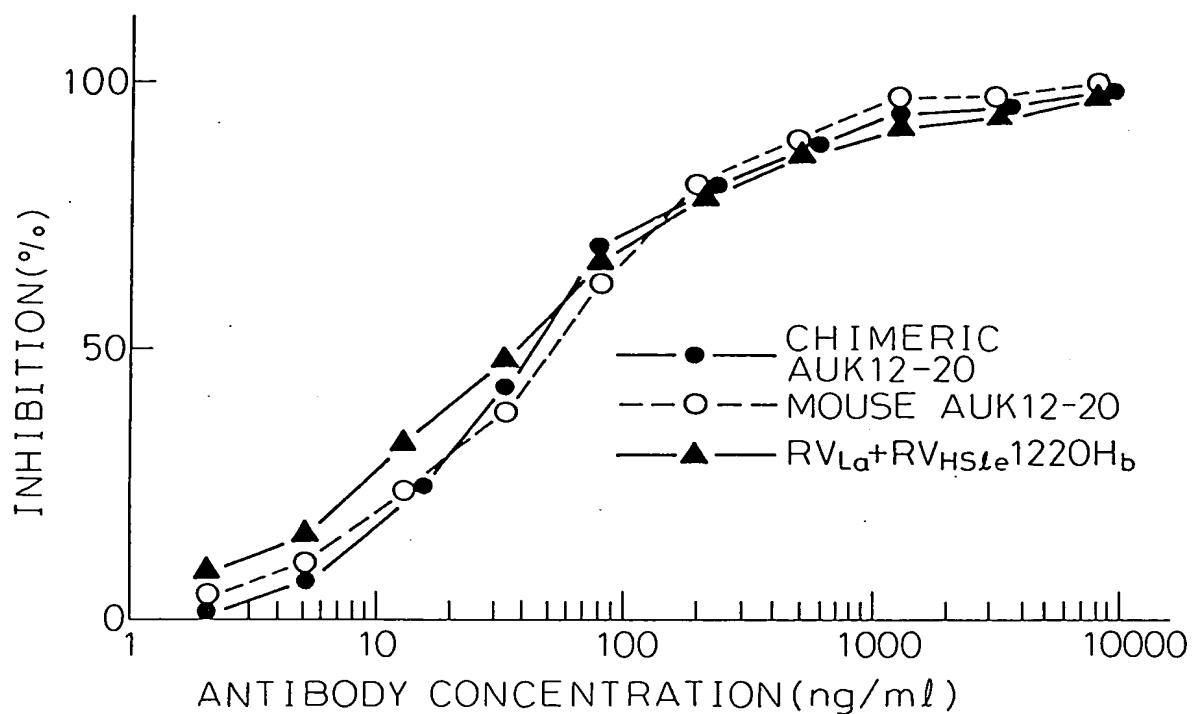
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Fig.21



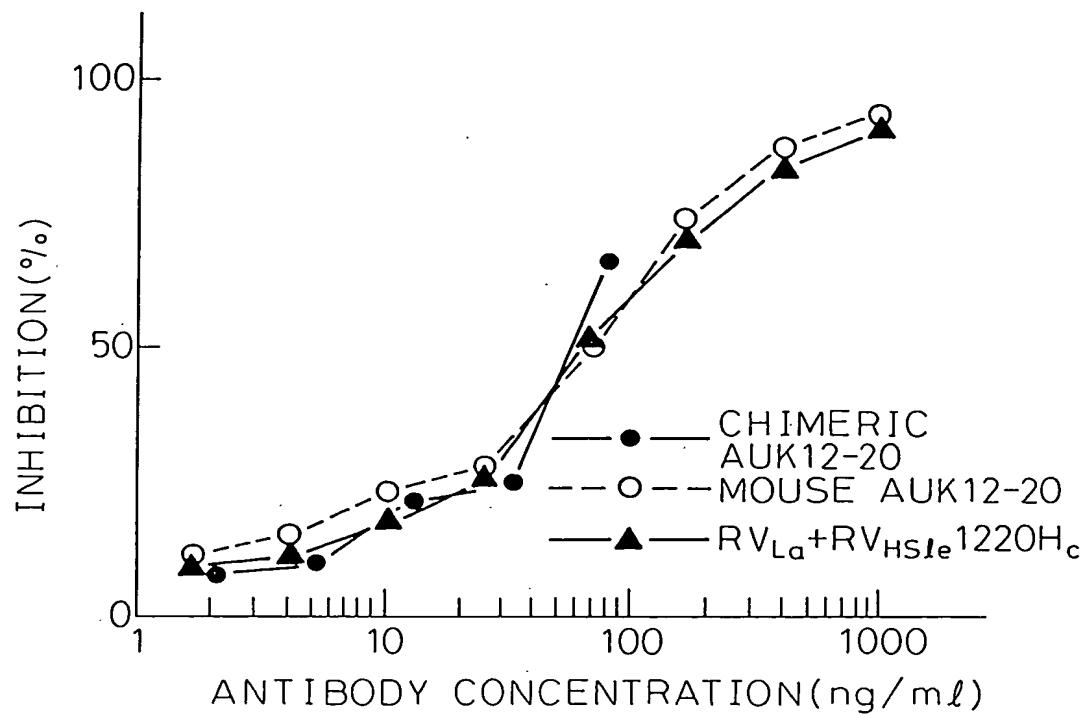
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Fig.22



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Fig.23



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Fig.24

